Cancer risk and hormonal changes in night shift workers

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ABSTRACT

Introduction: Recent human and animal data indicate that night shift work might increase the risk for cancer. Epidemiologic evidence is limited and has mostly focused on breast cancer while underlying mechanisms are still poorly understood. Furthermore chronotype an individual characteristic that describes the preference for activity in the morning or evening has not been taken into account in most previous research.

Aims: The main aim of this thesis was to evaluate the association between night shift work and breast and prostate cancer risk in a population based multi-case-control study in Spain (MCC-Spain). An additional aim was to study possible underlying mechanisms including the disruption of the daily production of melatonin and sex hormones.

Methods: Night shift work exposure was assessed with the use of lifetime occupational history for all MCC-Spain study participants, including detailed questions on night-shift schedules for each job held. We evaluated permanent and rotating night work as risk factors for different clinical types of prostate and breast cancer, taking into account individual chronotype. In a biomarker based study we compared daily rhythms of production of 6-sulfatoxymelatonin, the main metabolite of melatonin, and 16 sex hormones and metabolites in urine, between night and day workers of both sexes.

Results: Having ever worked at night was associated with an increased risk for prostate cancer, particularly for tumors with worse prognosis. Risk increased with duration of exposure and was higher among evening chronotypes. Having ever worked permanent or rotating night shift were also associated with an increased but not statistically significant risk for breast cancer compared to day workers. Risk was higher among women who worked for more than

15 years in permanent night shift and those who were exposed before their first full term pregnancy. Night shift workers had significantly lower 6-sulfatoxymelatonin levels and higher androgens and progestagens, while smaller differences were observed for estrogens. The peak time of 6-sulfatoxymelatonin and androgen production occurred later in night workers, compared to day workers.

Conclusions: Night shift work was associated with prostate cancer risk, particularly for tumors with worse prognosis. Night shift work was also associated with breast cancer risk, especially in women that started working at night before their first full-term pregnancy. Lower melatonin, higher sex hormone levels and mistimed hormone production are possible mechanisms that may partly explain the increased risk for hormone-related tumors in night shift workers.

RESUMEN

Introducción: Evidencia reciente en humanos y animales indica que el trabajo nocturno puede aumentar el riesgo de cáncer. La evidencia epidemiológica está limitada, sobre todo se ha enfocado en el cáncer de mama y los mecanismos involucrados no se han estudiado lo suficiente. Además, el cronotipo, una caracteristica individual que describe la preferencia por actividad a la mañana o la tarde, no se ha tenido en cuenta en los estudios previos.

Objetivos: El objetivo principal de esta tesis fue evaluar la asociación entre el trabajo nocturno y el cáncer de mama y próstata en un estudio poblacional de casos y controles en España (MCC-Spain). Un objetivo adicional fue estudiar posibles mecanismos como la disrupción de la producción de la melatonina y las hormonas sexuales.

Métodos: La exposición al trabajo nocturno se evaluó a través de la historia laboral de los participantes del estudio MCC-Spain, utilizando información detallada sobre los horarios de trabajo. Se evaluó el trabajo nocturno de modo permanente y rotativo como factor de riesgo para distintos tipos clínicos de cáncer de prostata y mama, teniendo en cuenta el cronotipo. En un estudio de biomarcadores se comparó el ritmo de la producción de la 6-sulfatoxymelatonina, el metabolito principal de la melatonina y de 16 metabolitos de hormonas sexuales en orina, entre trabajadores de día y de noche de ambos sexos.

Resultados: Haber trabajado de noche se asoció con un incremento de riesgo de cáncer de próstata, particularmente para los tumores con peor pronóstico. El riesgo de cancer de prostata fue más alto para exposiciones largas y en trabajadores con un cronotipo vespertino. El trabajo nocturno, permanente o rotativo, se asoció tambien con un mayor riesgo de padecer cáncer de mama, sin embargo, las diferencias no resultaron estadísticamente

significativas. El riesgo resultó mayor para mujeres con exposiciones más largas y para las mujeres que trabajaron de noche antes de tener su primer hijo. Los trabajadores de turno de noche tenían niveles de melatonina más bajos y niveles de andrógenos y progestágenos más altos que los trabajadores de turno de día, mientras diferencias mas pequeñas se detectaron en los niveles de estrogenos. La hora del pico de la produccion de la 6-sulfatoxymelatonina y de los androgenos era mas tarde en los trabajadores nocturnos que en los que trabajaban de dia.

Conclusiones: El trabajo nocturno fue asociado con un incremento del riesgo de cáncer de próstata, en particular para los tumores con peor pronostico. El trabajo de noche fue asociado tambien con el cancer de mama, en especial para las mujeres que habian trabajado de noche antes de tener su primer hijo. La supresión de la melatonina, el incremento de las hormonas sexuales y las alteraciones de los ritmos hormonales son mecanismos que pueden explicar en parte el incremento del riesgo de tumores hormono-dependientes en trabajadores de turno de noche.

PREFACE

Night shift work is one of the most prevalent occupational exposures, in numerous employment sectors, such as health, restaurants, transportations, communications and industry. It is predicted that night shift work will become even more frequent in the future as we move into a 24-h society. Exposure to night shift work results in alteration of sleep-wake cycles and desynchronization of human biological rhythms from the light–dark cycles, that has been associated with adverse health outcomes including cancer.

This thesis aims to assess the association between night shift work and breast and prostate cancer risk within a Spanish population-based case-control study and mechanisms using biomarkers of exposure in a cross-sectional study in Spain. The novelty of this study lies in the use of individual information on occupational history with detailed shift information that permitted a refined night shift work exposure assessment and the use of biomarkers of circadian disruption to assess possible underlying hormone related biological mechanisms of the described associations.

This thesis has been written at the Centre for Research in Environmental Epidemiology (Barcelona, Spain) between 2010 and 2014 and supervised by Prof. Manolis Kogevinas. It consists of a compilation of scientific publications in agreement with the regulation of the Doctoral Programme in Biomedicine of the Department of Experimental and Health Sciences at the Pompeu Fabra University. This thesis includes an abstract, a general introduction, a rationale, the objectives, the methods, the results (a compilation of four research publications and a commentary), an overall discussion section and final conclusions.

CONTENTS

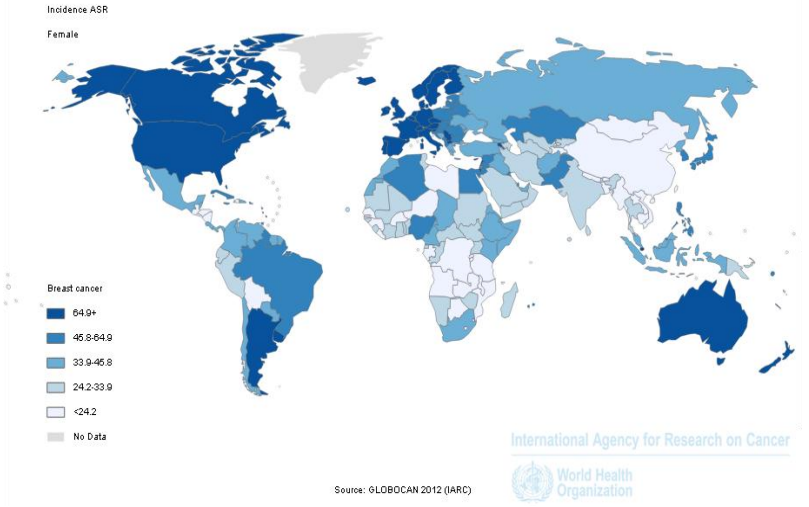
1. INTRODUCTION

1.1 Cancer Epidemiology

1.1.1 Breast cancer

Breast cancer is the most frequent tumor and the number one cause of cancer death in women worldwide (Ferlay et al., 2010; Ferlay et al., 2013). There is four to five-fold variations in rates worldwide, with the highest rates observed in Europe and North America and the lowest in Asia. Migrant studies show that women that move from low to high risk countries eventually adopt the rates of the host countries, indicating that international differences in breast cancer incidence are partly explained by environmental, occupational and lifestyle factors.

Figure 1.1.1 Breast cancer incidence among females (Globocan 2012)



A steady increase has been observed in breast cancer incidence over the last 100 years. Changes in reproductive factors (e.g use of oral contraceptives and hormonal therapy) and lifestyle (e.g. diet, physical inactivity, obesity) in addition to the increasing use of screening mammography are partly responsible for the observed

increase in incidence of breast cancer (Adami et al., 1998; Hankinson, 2008).

Around 5-10% of all breast cancers can be attributed to genetic factors (Bennett, 1999). Family history of breast cancer is an established risk factor for breast cancer and women with a first-degree relative with breast cancer have a relative risk of 1.5-3.0 compared to those whose first degree relatives were free of breast cancer (Greene, 1997). Several dietary factors have been suggested to affect breast cancer risk (Albuquerque et al., 2014). Among them alcohol intake has been most consistently, though modestly, associated with breast cancer risk, most likely through an increase of circulating estrogens (Tjonneland et al., 2007). Reproductive factors such as early age of menarche, late age of first full-term birth and low parity are well-known risk factors for breast cancer (Hankinson, 2008). Although the cause of breast cancer is not known, the mechanisms mediating the protection conferred by an early full-term pregnancy have been identified to reside in the breast itself, and to be modulated by endogenous and environmental exposures that might negatively affect this organ during specific windows in its development that extend from prenatal life until the first pregnancy (Russo & Russo, 2011). After menarche but before first pregnancy the breast has mainly undifferentiated ducts and alveolar buds (lobule types 1 and 2). Differentiation of epithelial cells is gradual and terminal differentiation (lobule 3 and 4) takes place after the first full-term pregnancy (Russo & Russo, 2008). The terminal differentiated cells have longer cycles and stay longer in resting phase G1, allowing more time for DNA repair, thus making breast epithelium more resistant to carcinogenic changes. Oral contraceptive use possibly increases risk for breast cancer while long-term hormone replacement therapy probably does, particularly the use of combined estrogen plus progestin therapies (Willett et al., 2000). The relationship between body mass index (BMI) and breast cancer is complex and varies depending on menopausal status. Obesity is inversely associated with breast cancer in premenopausal

women while it is positively associated to postmenopausal breast cancer (Adami et al., 1998; Weiderpass et al., 2004).

Sex hormones have been long-suggested to play an important role in breast cancer etiology. The risk of breast cancer increased with increasing circulating estrogen levels in a pooled analysis with data from nine prospective studies (Key & Allen, 2002). Subsequently more prospective studies have been published supporting these findings (Kaaks et al., 2005b). Risks were higher in women with estrogen and progestagen receptor positive tumors (Key et al., 2011; Missmer et al., 2004). Data are more limited and inconsistent among premenopausal women due to menstrual cycle hormonal fluctuations and related difficulties when sampling (Key et al., 2013). Estrogen metabolism has also been hypothesized to influence breast cancer risk, however only a few studies have evaluated this hypothesis (Willett et al., 2000). Androgens have been suggested to increase breast cancer risk directly, increasing the growth and proliferation of breast cancer cells, and indirectly through conversion to estrogens in fat tissue (Key et al., 2013). In postmenopausal women testosterone, as well as other androgens have been consistently associated with an increased breast cancer risk (Kaaks et al., 2005b; Zhang et al., 2013). In each of these analysis risk estimates persisted after adjusting for estradiol levels, showing some independent effects of circulating androgens on breast cancer risk. Among premenopausal women evidence is more limited, though consistent, with nested case-control studies showing a positive association between lifetime exposure to circulating androgens and breast cancer risk. (Eliassen et al., 2006; Kaaks et al., 2005a; Micheli et al., 2004). Progesterone plays an important role in breast tissue physiology and influence tumor development in animal studies (Brisken, 2013). High progesterone levels might increase breast cancer risk in humans since breast mitotic rate are higher in the luteal (high-progesterone) phase of the menstrual cycle. However in prospective studies results have not been consistent (Hankinson & Eliassen, 2007; Micheli et al., 2004).

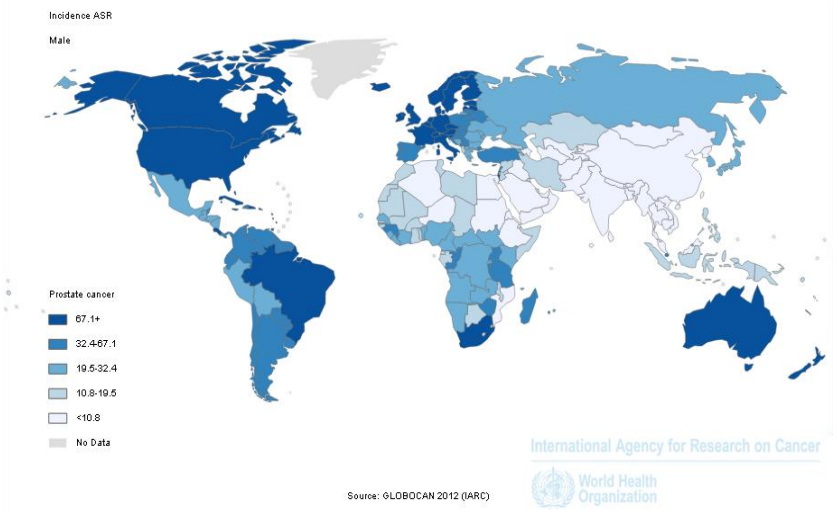
The idea that early life exposures might have an impact on future disease risk in childhood and adulthood is old,and it may also apply for breast cancer (Russo & Russo, 2011). In utero exposures might affect future breast cancer risk by alterations in normal breast development (Potischman & Troisi, 1999; Trichopoulos, 1990). Childhood, adolescence, early adulthood and pregnancy also play an important role in the breast development and hormonal balance (Hamilton & Mack, 2003). During these periods female breast tissue might be particularly vulnerable to environmental and occupational exposures.

1.1.2 Prostate cancer

Prostate cancer is the most common cancer in males with an increasing incidence worldwide (Ferlay et al., 2010; Ferlay et al., 2013; Siegel et al., 2013). The etiology of this tumor remains largely unknown and the only well-known risk factors include age, race, family history of prostate cancer and country of residence (Lorelei A Mucci, 2008). Hereditary factors explain 30-40% of prostate cancer risk and numerous genes associated with prostate cancer have been identified (Eeles et al., 2013). Therefore, no intervention strategy exists that could reduce the incidence of prostate cancer**.** Physical inactivity, smoking, sleep deprivation, vitamin D deficiency due to low sun exposure, and diet related factors, such as high meat and alcohol and low vegetable consumption and insulin-like growth factor-I (IGF-I) concentrations, are possible modifiable risk factors for prostate cancer, for which evidence is inconclusive (Lorelei A Mucci, 2008).

Androgens also play a key role in the normal growth and functioning of the prostate. Prostate cell division is strongly influenced by certain steroid hormones, including testosterone and dihydrotestosterone.

Figure 1.1.2 Prostate cancer incidence among males (Globocan 2012)



Evidence from animal studies shows that large amount of androgens can induce prostate cancer in rodents (Brown et al., 1979). Lifetime exposure to increased androgen levels may increase risk for prostate cancer and antiandrogenic therapies are commonly used against prostate cancer (Sharifi et al., 2005). Most epidemiologic studies do not support the hypothesis that high levels of circulating androgens correspond to an increase of prostate cancer (Roddam et al., 2008), while a possible association with estrogen metabolites have been hypothesized (Kosti et al., 2011) as well as possible ivesrse association of the estrogen/androgen ratio and aggressive prostate cancer risk (Black et al., 2014). Case-control studies have been criticized for possible reverse causality given that prostate cancer may modify sex hormone levels. Furthermore many of the sex hormones have circadian rhythms and epidemiological studies have poorly accounted for this by matching the timing of sample collections or restricting samples in the same time-in-day window (Bao et al., 2003; Yie et al., 1990). A single blood-measurement is probably not very reliable for characterization of an individual's hormonal profile.

In utero exposure to sex hormones has been hypothesized to relate with adult cancer risk. Anogenital distance (AGD) is the distance from the anus to the genitalia, and is currently being used as a measure of fetal androgen exposure. AGD tracks through life and is a highly reliable anthropometric measurement (Papadopoulou et al., 2013). Findings of recent studies have linked AGD length and reproductive parameters in adulthood. Decreased AGD was found among prostate cancer patients compared to population controls in the MCC-Spain study (Castano-Vinyals et al., 2012).

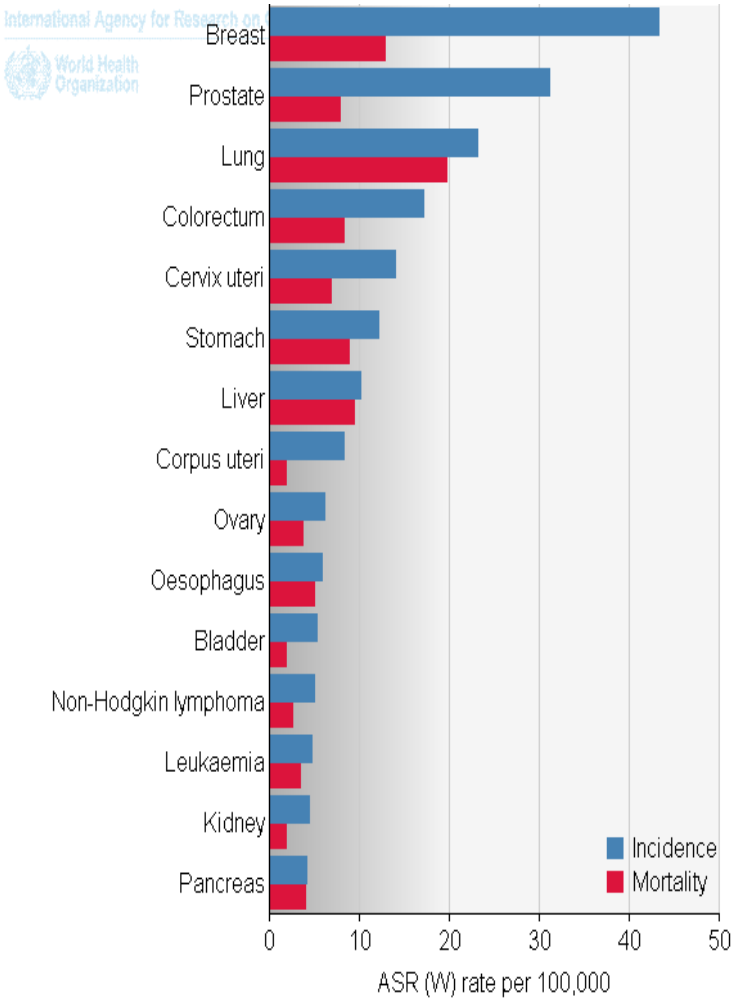
1.1.3 Breast and prostate cancer: similarities

Breast and prostate cancer are the two most common tumors in men and women combined, accounting for over one fourth of all incident malignancies in the western countries (Figure 1.1.3.1). The etiology of both these tumors is complex and remains rather enigmatic for epidemiologists and clinicians (Hankinson, 2008; Lorelei A Mucci, 2008). Despite the extensive research on the identification of risk factors associated with their incidence only part of the risk has been explained so far. They both have a strong heritable component compared to other cancers. They also both depend on sexual hormones for their development. Finally they both show substantial geographical variation and increasing incidence in developing countries indicating that environmental, occupational and lifestyle factors are important for their etiology.

Over the last 15 years, there is increasing evidence that both these two endocrine cancers are associated with recent changes in human behaviors related with the increasing use of electrical light (Hansen, 2001; Schernhammer et al., 2001; Stevens, 1987). People living in larger cities with higher levels of indoor and outdoor light have an increased risk of breast and prostate cancers compared to people living in rural areas (Kloog et al., 2009; Kloog et al., 2010). It has been hypothesized that the societal adoption to 24-hours activity in daily life, has increased exposure to light-at-night, decreased sleep

duration and diminished exposure to daily sunlight (vitamin D which possibly protects against these cancers) (Stevens et al., 2013). Night shift work is one of the most common occupational exposures in the developed countries and night shift workers represent subjects with extreme exposure to light at night and disruption of their sleep-wake patterns and daily biological rhythms (Arendt, 2010; Costa, 2010).

Figure 1.1.3 Incidence and mortality rates for worldwide breast and prostate cancer in men and women combined (Globocan 2012)



1.2 Shift work

1.2.1 Definitions of shift work

The International Labour Organization defines shift work as "a method of organization of working time in which workers succeed one another at the workplace so that the establishment can operate longer than the hours of work of individual workers" (International Labour organization, 1990). In the scientific literature the term "shift work" has been widely used and includes any work that takes place outside the standard daylight hours (7/8 a.m. to 5/6 pm).

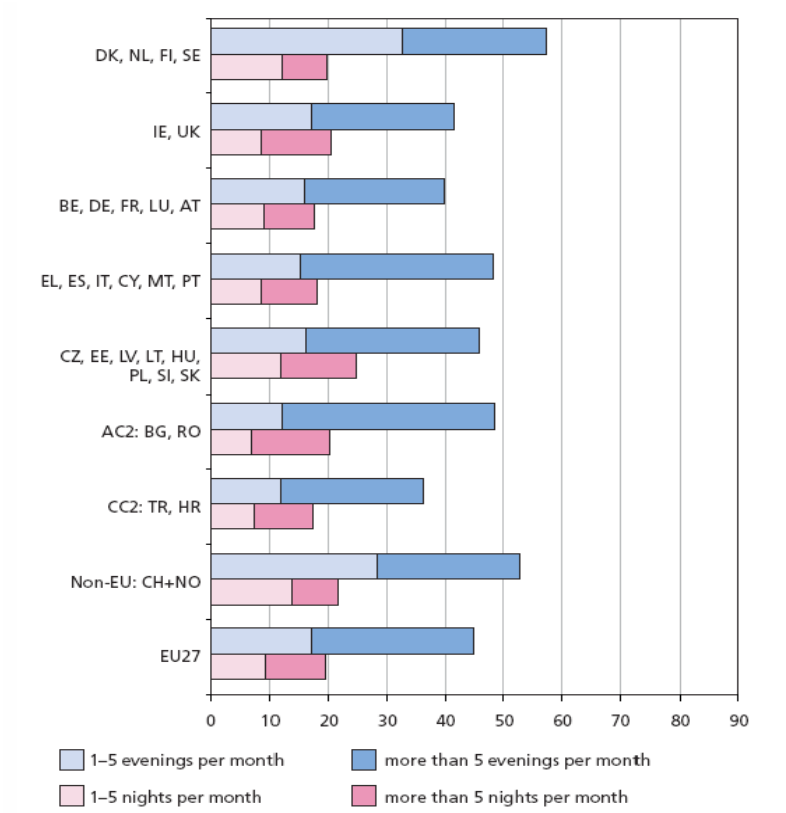
1.2.2 Prevalence of shift work

Shift work is one of the most frequent occupational exposures in the industrialized part of the world with 20-30% of the population engaged in some kind of unusual working hours. This number is expected to increase according to predictions of a progressive move towards a 24/7 society (Parent-Thirion A., 2012).

1.2.3 Shift work patterns

Shift work schedules are complex; they can be regular or irregular, with changes in their organization. A shift schedule can be permanent (always the same shift) or rotating (rotation among different shifts: morning, evening and/or night). Shift work may include or not night work. The definition of night work varies across countries and it ranges from 8-10 p.m to 5-7 a.m (IARC, 2010).Rotating shift systems differ largely with respect to shift cycle length, duration of shifts, start and finish time of the periods, speed of shift rotation-depending on the number of consecutive shifts worked in each shift (fast rotation: 1-3 days, medium rotation: every week, slow rotation: 15-30 days), direction of shift rotation (clockwise: morning, afternoon, night vs counter-clockwise: afternoon, morning, night) and number of rest days between shifts.

Figure 1.3.1 Prevalence of shift work that includes night work, by country in Europe in 2005 (4th EU Survey on working conditions)



Country codes

EU15	15 EU Member States prior to enlargement in 2004		
NMS	10 new Member States that joined in 2004		
EU25	15 EU Member States, plus the 10 NMS		
EU27	25 EU Member States, plus the AC2		
AC2	Two countries that joined the European Union in 2007: Bulgaria and Romania		
CC2	Two candidate countries for membership of the EU: Croatia and Turkey		
AT	Austria	LU	Luxembourg
BE	Belgium	MT	Malta
BG	Bulgaria	NL	Netherlands
CY	Cyprus	PL	Poland
CZ	Czech Republic	PT	Portugal
DK	Denmark	RO	Romania
EE	Estonia	SK	Slovakia
FI	Finland	SI	Slovenia
FR	France	ES	Spain
DE	Germany	SE	Sweden
EL	Greece	UK	United Kingdom
HU	Hungary	HR	Croatia
IE	Ireland	NO	Norway
IT	Italy	CH	Switzerland
LV	Latvia	TR	Turkey
LT	Lithuania		

Different shift systems are used depending on the occupational sector (Sallinen & Kecklund, 2010). Industry is usually arranged in 3-shift systems, using fixed start and stop times and rotation of different speeds. In the transport sector, schedules are often irregular concerning the number of consecutive shifts, the duration of the shift and start and stop times. In the health care-sector both permanent and rotating shift schedules are operated. In the service sector split shifts and permanent night shifts are used while in the leisure sector shifts cover mostly the late afternoon and night hours .

Night shift work has been associated with a wide range of acute health effects (e.g. sleep and digestive problems) but also a wide range of chronic diseases, including cardiovascular disease, metabolic syndrome, diabetes and cancer (Wang et al., 2011). The evidence on the association between night shift work and cancer risk will be discussed in-depth in continuation, as well as some of the main proposed underlying biological mechanisms.

1.3 Carcinogenicity of shift work

In 2007 the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) evaluated the carcinogenicity of shift-work (IARC, 2010; Straif et al., 2007). They concluded that "shift-work that involves circadian disruption is probably carcinogenic to humans" (Group 2A). This evaluation was primarily based on "sufficient evidence in experimental animals for the carcinogenicity of light during the daily dark period". Also an increasing number of epidemiological studies in the last years suggest a possible association between the disruption of normal circadian rhythm and risk for developing cancer. Most of these studies have focused on female night-shift workers and showed a positive association between night work and breast cancer risk. Although breast cancer is the cancer most commonly studied tumor, recently a few studies have considered the effect of night-shift work on other types of endocrine cancer, such as prostate, and endometrial cancer (Viswanathan et al., 2007) and some reports have shown data on a wide range of tumor localizations, including colorectal cancer (Kolstad, 2008; Parent et al., 2012; Wang et al., 2011). In continuation the evidence for the association between night shift work and the two tumors evaluated in this thesis, breast and prostate cancer, will be described.

1.3.1 Night shift work and breast cancer risk

The strongest evidence to date shows that women who work at night have a higher risk of breast cancer. In 2005, Megdal et al. carried out a meta-analysis of the data on night shift work and breast cancer (including studies of airline cabin crews and night shift workers) and reported a summary relative risk (RR) of 1.48 (95% confidence interval (CI) 1.36–1.91) for all studies combined (Megdal et al., 2005). All studies published from 1996-2007 were included in the subsequent evaluation of the IARC and are described in more detail below.

In a nationwide population-based case-control study, in Denmark including 7035 female cases between 30 and 54 year-old an increased risk of breast cancer associated with shift-work was reported (Hansen, 2001). The OR for breast cancer among women who had ever worked at night at least half a year and at least 5 years prior to diagnosis was 1.5 (95% 1.2-1.7), adjusted for reproductive history and socioeconomic status. Exposure to artificial light during night and night-shift was studied in a case-control study of breast cancer in Seattle, USA, including 813 cases and 793 controls, all aged 20-74 years, frequently matched according to 5-year age groups (Davis et al., 2001). In this study night work exposure was defined as at least one graveyard (beginning work after 7p.m. and ending before 9a.m.) shift per week in the 10 years before diagnosis. The estimated relative risk for breast cancer after ever working graveyard shift was 1.6 (95% CI: 1.0-2.5). The above results were, to a certain extent, confirmed in two large prospective studies including nurses participating in the Nurse's Health Study, engaged in rotating night shifts (Schernhammer et al., 2006b; Schernhammer et al., 2001). In a nested case-control study performed within a cohort of Norwegian nurses an association between duration of night work and breast cancer risk was found (p=0.01), with a RR for working 30 or more years of night shift was 2.21 (95%CL, 1.10-4.45) (Lie et al., 2006). Elevated breast cancer risk (OR=4,3; 95%IC 0,7-26) after adjusting for duration of employment was also seen among postmenopausal >50 years old radio and telegraph operators exposed to shift work in a case-control study in Norway (Tynes et al., 1996). Evidence from the GENICA case-control study in Germany, assessing shift work information for 857 breast cancer cases and 892 controls, enrolled between 2000 and 2004, showed that long-term work was associated with a modestly but not significantly increased risk, while having ever worked was not (Pesch et al., 2010).

A case-control study conducted in Long Island did not observe an association between night work and breast cancer (any evening or

night shift versus none) (O'Leary et al., 2006). However in this study 35.7% of cases and 36.9% of controls reported any "evening or overnight shift work", proportions which were considered as remarkably high. Furthermore night-workers in this study tended to be younger and more frequently premenopausal compared to other studies. Schwartzbaum et al. also found no increase in risk in female breast cancer in a retrospective register based ecologic cohort study (Schwartzbaum et al., 2007). However this study included a rather small proportion of women working night shifts and the four most common occupations that fell into the shift work classification were rather unusual (crane and hoist operators, delivery women in paper and paper products manufacturing, printing and publishing industries, midwives).

Since the IARC classification, there have been more epidemiological studies, with refined exposure assessments and on a variety of occupations in different populations. These more recent studies include both case-control and cohort studies and have mostly supported an association between night shift work and breast cancer (Fritschi et al., 2013; Grundy et al., 2013; Hansen & Lassen, 2012; Hansen & Stevens, 2012; Knutsson et al., 2013; Lie et al., 2011; Menegaux et al., 2013), but also reported on the no association (Pronk et al., 2010). A few reports from existing studies have also been published analyzing night shift work with respect to different clinical subtypes of breast cancer and particularly evaluating risk by estrogen and progestagen receptor status (Lie et al., 2013; Rabstein et al., 2013).

Overall the evidence from epidemiological studies is mostly positive notwithstanding inconsistencies and lack of clear dose-response (Bonde et al., 2012). Several systematic reviews and meta-analyses have been performed, on the association between night shift work and breast cancer risk, four of them published in 2013 (Ijaz et al., 2013; Jia et al., 2013; Kamdar et al., 2013; Kolstad, 2008; Wang et al., 2013). Two of the four recent reviews concluded

that the epidemiologic evidence is overall weak or insufficient (Ijaz et al., 2013; Kamdar et al., 2013) whereas the other two supported the hypothesis (Jia et al., 2013; Wang et al., 2013) . Jia et al. estimated an overall relative risk of 1.20 (1.08–1.33), and from the 'higher-quality' studies a risk of 1.40 (1.13–1.73). Even though inconclusive between them, all reviews highlighted the methodological limitations of the existing studies and the heterogeneity between studies in methods including basic definitions of exposure.

Finally, it also interesting that a higher incidence of male breast cancer was also observed in occupations characterized by shift work and night work in a 45 year follow up of cancer incidence data by occupational category in the Nordic countries, while the hypothesis was not confirmed in women (Pukkala et al., 2009).

1.3.2 Night shift work and prostate cancer risk

Most epidemiological studies have focused on female breast cancer and evidence on other tumor sites is scarce. A potential effect of night shift work, light at night and sleep disruption on prostate cancer incidence has been recently suggested and evidence critically reviewed (Sigurdardottir et al., 2012).

An increased prostate cancer risk has been observed among night workers in four studies (Conlon et al., 2007; Kubo et al., 2012; Kubo et al., 2006; Parent et al., 2013). In a Japanese cohort study rotating night shift work was associated with a 3-fold increase (RR 3.0 ; 95% CI 1.2, 7.7) and fixed night work with a 2-fold non-significant increase (RR 2.3, 95% CI 0.6, 9.2) in risk (Kubo et al., 2006). A limitation of this study was the small number of porstate cancer cases (in total 11 exposed cases: 7 with rotating and 3 with fixed night shift shcedules) and that exposure classification was based on the longest job. In a subsequent publication of the same group, including 17 prostate cancer cases, rotating shift workers

were at an increased but non-significant risk (RR 1.79, 95% CI 0.57, 5.68) (Kubo et al., 2012). In a case-control study in Canada an increased risk for prostate cancer was found among full-time rotating shift workers (1.19, 95% CI 100, 1.42), although estimates were only adjusted for age and family history of prostate cancer (Conlon et al., 2007). In a recent multi-case control study fixed night shift work was associated with prostate cancer independent of duration or timing of epxosure (2.77; 95% CI 1.96, 3.92) (Parent et al., 2012).

Not all studies have showed evidence of an association between shift work and prostate cancer. In a swedish cohort study there was no increase of prostate cancer risk among shift workers, compared to the general population, however the exposure assessment was rather crude, based on job-title and comparisons of industries with more than 40% of shift workers with occupations vs less than 30% of shift work, therefore exposure misclassification was very likely (Schwartzbaum et al., 2007). Furthermore shift work did not have to include night work. In a large porspective study in the USA no association was found between rotating or fixed night shift work and fatal prostate cancer, however relevant exposure information, such as duration and frequency were missinng (Gapstur et al., 2014) . Finally, in an industry based cohort study in Germany shift work was not associated with prostate cancer through record linkage with the cancer registry (HR 0.93, 95% CI 0.71–1.21), after adjustment for age, smoking status, job level, and employment duration (Yong et al., 2014).

Sleep duration, a proxy for hours spent in darkness, has also been associated with prostate cancer risk in two studies (Kakizaki et al., 2008; Sigurdardottir et al., 2013). In a cohort of Japanese men sleep duration was inversely associated with the risk of prostate cancer (Kakizaki et al., 2008). The association between short sleep duration and prostate cancer risk was stronger for advanced or metastatic disease.

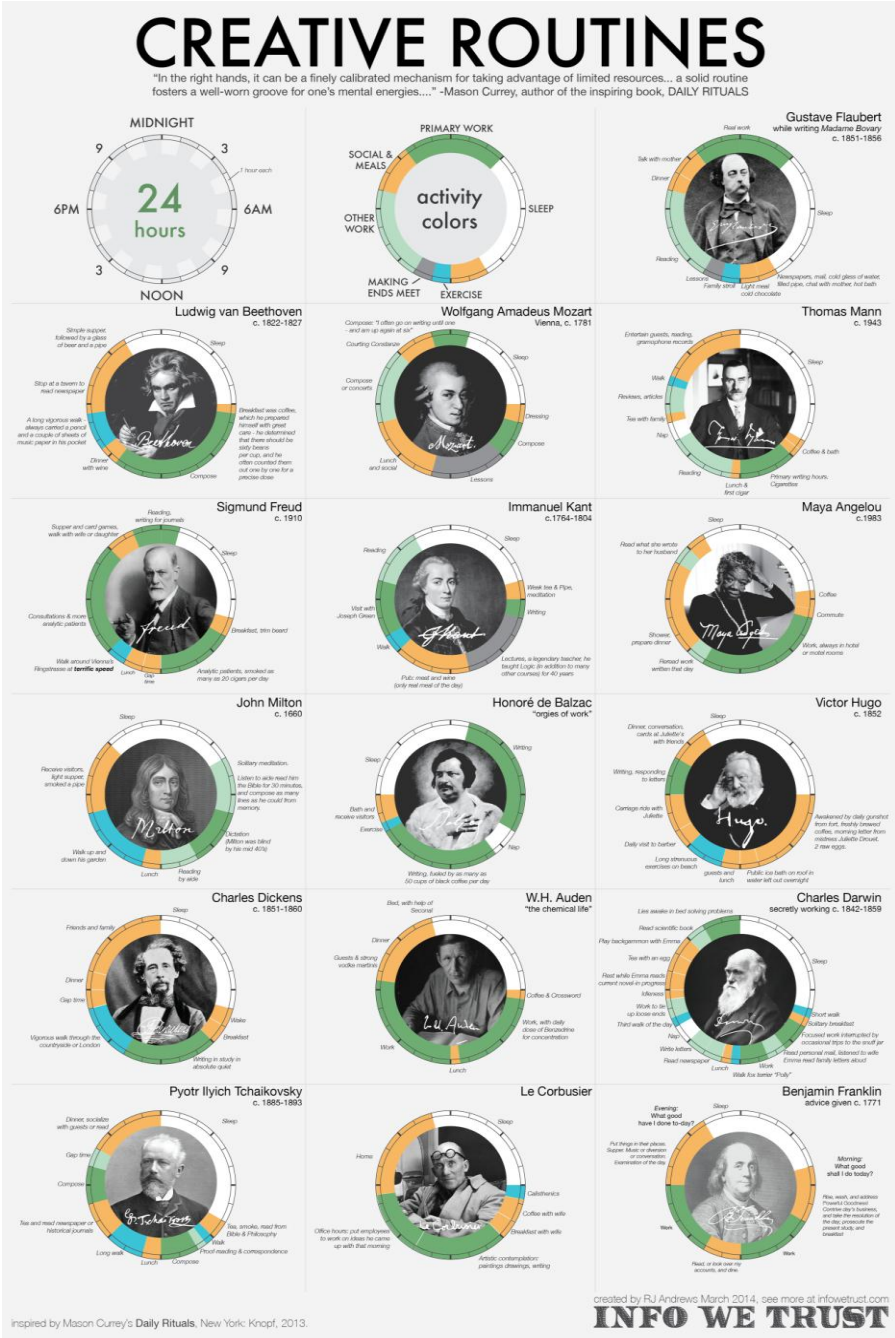
Finally, an ecological study evaluated light at night levels provided by satellites with respect to cancer risk using age-standarized incidence rates of prostate, lung and colon cancer, among men residing in 164 countries. They showed that only prostate cancer was significantly correlated with light at night levels provided by satellites at the country level (Kloog et al., 2009).

1.3.3 Limitations of epidemiological studies

An important limitation that made interpretation and comparison of results from previous studies very difficult was the lack of uniformity in shift work definitions used across studies. In fact almost all existing studies have used different definitions. Some studies had individual while other register-based information, some evaluated permanent whereas others rotating schedules and not all studies had information on duration and/or intensity (nights/month) of night shift work over working lifetime. An IARC consensus report stressed the need for a more refined exposure assessment in order to capture aspects of shift work schedules that might be relevant for cancer risk and preventive actions with particular emphasis on: (1) type of shift work (permanent versus rotating shifts), (2) duration (years of night work) and (3) intensity (number of night shifts/month) (Stevens et al., 2010). Incomplete adjustment for confounding was a main limitation of the earlier studies, that had not performed a minimal adjustment for basic confounders such as age, socioeconomical status, parity, age first child and BMI. Most of the later studies, especially case-control studies, adjusted for several known or suspected risk factors for breast cancer but there was not much evidence for confounding. Most previous studies were performed among a single profession, nurses or flight attendants. More studies are needed on a variety of other occupations that include night shift work in order to extrapolate the associations to all night shift workers.

Only a few previous studies have taken into account individual characteristics, related to suceptibility to night shift work, in their assesments (Bonde et al., 2012). Individual chronotype is a human attribute with genetic basis that reflects the time of the day that functions are active (Roenneberg et al., 2007). Chronotype correlates with diurnal preference an attribute reflecting personal preference for activities in the morning or evening (Roenneberg et al., 2003). In a given population, chronotypes range from early to late types—the colloquial "larks" and "owls". Larks tend to wake up at an early hour and find it difficult to stay up late in the evening, while extreme owls are almost nocturnal in their activity, going to bed in the early morning hours and sleeping late into the day. Subjects differ substantially with respect to their chronotype or personal preference for timing of sleep and activity (Figure 1.3.1). It has been suggested that diurnal preference and chronotype may affect shift work adaptation and that evening types (subjects with a later melatonin peak) may adapt faster to night shift work (Saksvik et al., 2011). For this reason it has been hypothesized that chronotype may modify the risk for cancer since it may associate to the capacity of night workers to adapt to non-day work schedules (Erren, 2013). One recent study evaluated chronotype in conjunction with night shift work and breast cancer risk (Hansen & Lassen, 2012), while chronotype has also been suggested as an independent risk factor for cancer. One study found a higher breast cancer risk for evening types while the other moderate increase among neither types (Hansen & Lassen, 2012; Ramin et al., 2013). However existing studies evaluated chronotype using a single question which might not appropriately capture chronotype. A validated questionnaire, the Munich Chronotype Questionnaire (MCTQ), exists for a more precise quantitative chronotype assesment (Roenneberg et al., 2003). No previous study has evaluated the association between chrononotype and prostate cancer or has examined effect modiffication of the night work-prostate cancer risk association by chronotype.

Figure 1.3.1 Interindividual variability in diurnal preference and sleep-activity patterns.



1.4 Mechanisms

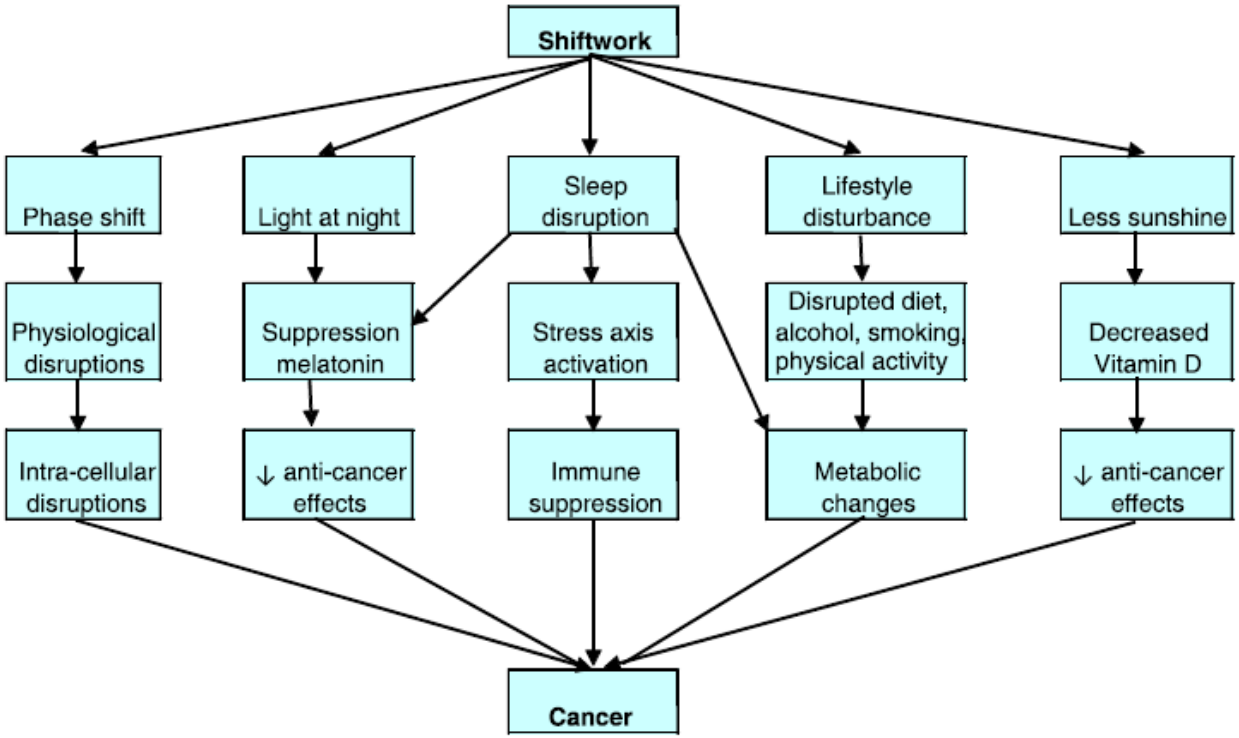
There have been several biological plausible mechanisms to explain the association between night shift work and cancer (Figure 1.4.1). It is suggested that subjects working at night-shifts are exposed to artificial light at night (LAN) and experience a suppression of the normal melatonin production, a possible increase in sex hormones, an alteration of sleep patterns and deregulation of circadian genes, changes that may be involved in cancer-related pathways. In continuation some of the main mechanisms will be described.

1.4.1 Circadian disruption

Circadian rhythms

All life on Earth, from cyanobacteria to humans, have adapted to the 24-hour light-dark cycle dictated by the Earth's rotation around its own axis. Internal body clocks with a near (*circa*) 24 hour (*diem*) periodicity impose rhythm on systemic and cellular human processes, such as body temperature, heart rate, hormone production, metabolism, immune function and gene expression (Figure 1.4.1.1). Circadian rhythms are internally generated, regulated by a central endogenous 'clock' in the hypothalamic suprachiasmatic nucleus (SCN) in the brain. Peripheral clocks exist in virtually all other organs and tissues, including the heart, liver and blood cells (Storch et al., 2002). Circadian rhythms have the ability to be reset or entrained by a stimulus (zeitgeber=time giver, in German) to a new rhythm. The most important synchronizer of the circadian system is light (Czeisler, 1995; Czeisler et al., 1986). Synchronization to the 24-hour light-dark cycle occurs primarily through daily resetting via environmental light exposure. The effect of light depends on characteristics of light such as intensity, duration, timing of exposure and wavelength of the light. Other non-photic zeitgebers include timing of meals (Stephan, 2002), physical activity (Barger et al., 2004) while social cues (e.g. knowledge of clock time) does not influence entrainment (Middleton et al., 1996).

Figure 1.4.1 Mechanisms involved in the association between night shift work and cancer risk (Fritschi et al., 2011)



Light at night

The invention of electrical lighting over 100 years ago, and its increasing use, has led to a progressive light pollution (Figure 1.4.1.2), shortening of the dark period and sleep and an increase in non-day activities. Almost everybody is exposed to light at night and its possible effects on the circadian system, sleep-wake cycles and diet patterns. Current human activities (use of bright screens, mobile phone and TV) during late evening, night and early morning are in contrast to the millions of years of evolutionary experience of days with bright light exposure and activities from about dawn to dusk followed by dark nights with sleep and rest. Therefore it has been suggested that the increasing use of electric light over the biological night might have porfound effects on the organization of their circadian system.

Circadian disruption

Circadian disruption is defined as the desynchronization or decoupling of internal circadian rhythms relative to ambient/dark/light cycle and the desnchronization of the SCN from the peripheral clocks. Night shift workers experience extreme decoupling of sleep-activity patterns from the natural light-dark cycle and extreme exposure to light at night and circadian disruption. With night shift work the central clock (SCN) shifts to adapt to the new light dark cycle and desynchronizes from the peripheral clocks in organs and cells, that results in circadian disruption (internal desynchronosis) also known as jet-lag. If a night worker did not switch to diurnal orientation on his/her free days, eventually the peripheral rhythms would also adapt and in that case the internal rhythms would be synchronized between them but would be desynchronized with the environment (external desynchronosis) (Monk, 2000).

Figure 1.4.1.1 Circadian rhythms over 24 hours (Credit:Wikipedia)

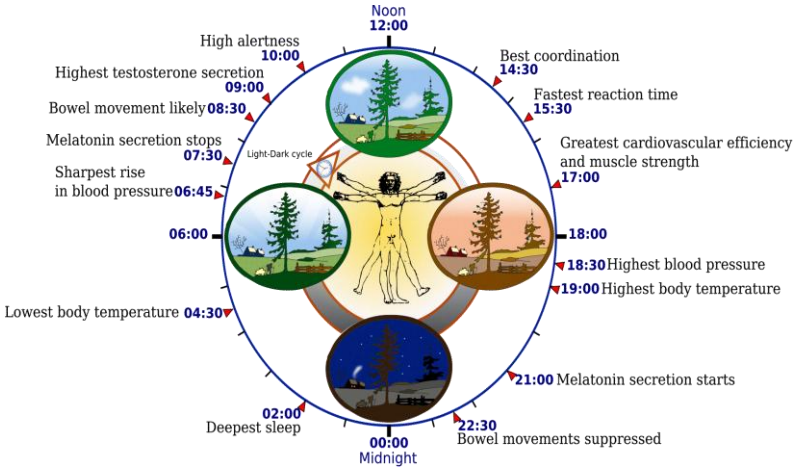


Figure 1.4.1.2 Satellite image of Europe by night (Credit: NASA)

There is increasing evidence for an association between circadian disruption and genetic driving machinery of cancer (Savvidis & Koutsilieris, 2012). Circadian genes are responsible for maintaining the 24-h circadian rhythms but also for a variety of cancer-related pathways including regulation of sex hormone levels (Stevens, 2005; Zhu et al., 2005) . The core circadian genetic loop at present is thought to consist of 10 genes, also known as "clock genes", yet controlling about 10% of the whole genome (Takahashi et al., 2008). Evidence suggests that there are interconnections between circadian gene function and cell-cycle regulation (Hunt & Sassone-Corsi, 2007). Loss of cell-cyle control is a key event in the carcinogenic process that may lead to a transformed cell and the initiation of a later diagnosed cancer. There is plenty of experimental evidence describing how circadian disruption links to cancer risk through alteration of clock genes and the desynchronization of the master from peripheral clocks (Haus & Smolensky, 2013; Storch et al., 2002; Zhu et al., 2005; Zhu et al., 2009) . Chronic disruption of clock gene expression that leads to cell cycle deregulation could increase DNA damage replication errors and resulting mutations (Sancar et al., 2010).

Finally there is some evidence that circadian genes express differently in breast cancer tissue compared to surrounding healthy tissues (Chen et al., 2005). Hypomethylation of the CLOCK promoter and hypermethylation of the CRY2 promoter was also found in peripheral blood lymphocytes of breast cancer cases compared to controls (Hoffman et al., 2010a; Hoffman et al., 2010b). Similar differences were found between night and day workers in a subsequent study comparing methylation patterns in the same regions of these two genes (Zhu et al., 2011). More evidence is needed before we can conclude on the possible role of circadian genetic susceptibility for cancer, gene-environment interactions with night shift work or the role of epigenetic mechanisms in cancer related pathways.

1.4.2 Hormonal changes

1.4.2.1 Melatonin

The "melatonin hypothesis"

One of the first proposed and most cited mechanism for the association between night shift work and cancer risk is the so called "melatonin hypothesis" (Stevens, 1987). According to this hypothesis, night shift workers experience light-induced suppression of their normal melatonin production which may in turn increase cancer risk. Although human experimental evidence support the suppression of melatonin after light exposure (Bojkowski et al., 1987; Lewy et al., 1980; Zeitzer et al., 2000), observational studies among night shift workers has not been as consistent. Some but not all field studies have showed lower melatonin levels related to night shift work, and only a few used objective light measurements (Borugian et al., 2005; Burch et al., 2005; Davis et al., 2012; Dumont et al., 2012; Grundy et al., 2009; Grundy et al., 2011; Hansen et al., 2006; Langley et al., 2013; Peplonska et al., 2012; Schernhammer et al., 2006a; Schernhammer et al., 2004; Yamauchi, 2004). Furthermore the majority of the existing studies used a single or a few urine samples for melatonin assessment. This made the interpretation of the observed differences difficult, due to possible confounding by circadian stage. In most studies, only absolute melatonin levels were assessed and not peak time of production, representing a major gap in existing literature. In continuation the physiology of melatonin, its use as a biomarker of circadian disruption in epidemiologic studies and the evidence for its oncostatic role are described.

Physiology of melatonin

In mammals, the indoleamine hormone melatonin (N-acetyl-5-methoxytryptamine) is synthesized primarily in the pinealocytes of the pineal gland, in a circadian manner (Arendt, 1995). In humans melatonin is produced over the daily dark period and peaks between 02:00-04:00h (Figure 1.4.2.1). The melatonin rhythm is controlled

exclusively by the SCN. Light controls the body clock by acting on the SCN. Melanopsin receptors in the eyes' retina respond to the presence of light by transmitting signals to the SCN. Light-induced activation of the SCN prevents the pineal gland from producing melatonin. Light suppresses melatonin in a dose–response and wavelength-dependent manner (Figure 1.4.2.2). Blue light (peak light sensitivity: 460-480 nanometers) is more disruptive for the circadian system and melatonin rhythm (Thapan et al., 2001). Melatonin suppression depends not only on light intensity and wavelength, but also on the duration, timing of light exposure, and recent light exposure history (Brainard et al., 2001; Lockley et al., 2003; Thapan et al., 2001; Zeitzer et al., 2000). Some dietary and lifestyle factors may also affect melatonin production (Zawilska et al., 2009). Alcohol consumption reduces melatonin in a dose-response manner, smoking increases levels and caffeine delays its clearance, increasing its plasma concentrations (Schernhammer et al., 2004; Stevens et al., 2000; Ursing et al., 2005). Changes in body posture may also affect melatonin levels (Arendt, 1995). Some drugs affect melatonin synthesis and metabolism. Adrenergic b-blockers and benzodiazepines (diazepamen, alprazolam) decrease melatonin while the selective serotonin reuptake inhibitor (SSRI) fluvoxamina and other antidepressants such as monoaminase oxidase inhibitors (MAOI) increase melatonin levels (Skene et al., 1994; Wirz-Justice & Arendt, 1980).

Figure 1.4.2.1 Normal pineal melatonin production over 24 hours

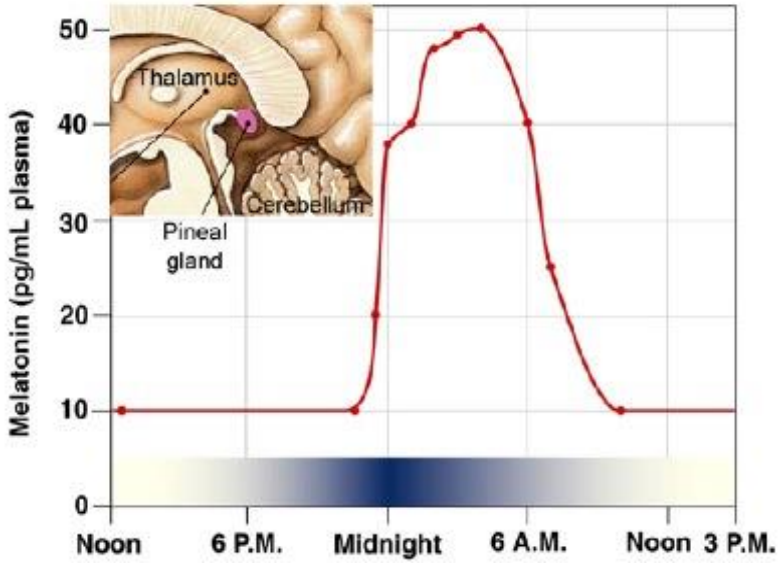
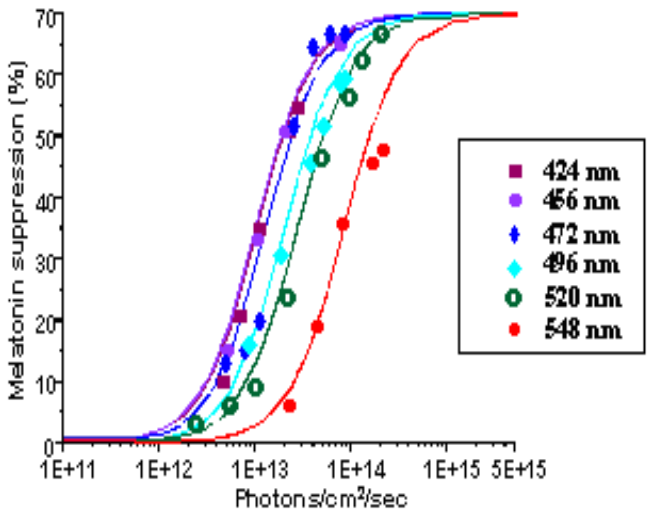


Figure 1.4.2.2 Melatonin suppression with increasing light intensity for different wavelengths of light (Thapan et al., 2001)



Melatonin: a biomarker of circadian disruption

Melatonin is considered the best biomarker of circadian regulation and dysregulation (Mirick & Davis, 2008). An advantage of the melatonin rhythm compared to other markers such as cortisol, core body temperature and heart rate is that it is more robust in the presence of external influences, such as food intake, or stress. Plasma melatonin has a short half-life and is rapidly metabolized in the liver. Hourly plasma melatonin measurements would perfectly capture its rhythm. However this is only possible in controlled settings (sleep labs). The measurement of the primary metabolite of melatonin, 6-sulfatoxymelatonin (aMT6s) excreted in urine allows on a non-invasive monitoring of the pineal function and circadian phase (Arendt et al., 1985). Urinary aMT6s excretion is a good indicator of plasma melatonin secretion (Cook et al., 2000). Repeated aMT6s urine measurements are an adequate tool in epidemiological studies for studying circadian disruption in shift workers.

Melatonin and cancer

An overwhelming amount of research has taken place on the cancer-protective role of melatonin. In humans, lower levels of melatonin have been found in breast cancer patients compared to controls, in three out of five prospective nested case-control studies, supporting partly the hypothesis on an inverse association between melatonin levels and breast cancer risk. Some inconsistencies have been attributed to study limitations related to melatonin sampling procedures and timing. (Schernhammer et al., 2010; Schernhammer et al., 2008; Schernhammer & Hankinson, 2005; Schernhammer & Hankinson, 2009; Travis et al., 2004). A recent study found that lower levels of melatonin were associated with an increased risk for advanced prostate cancer risk in men (Sigurdardottir et al., 2014).

Melatonin has well known oncostatic properties as an anti-oxidant, immuno-modulator and anti-mitotic agent (Vijayalaxmi et al., 2002). In vivo experiments show that both constant light exposure

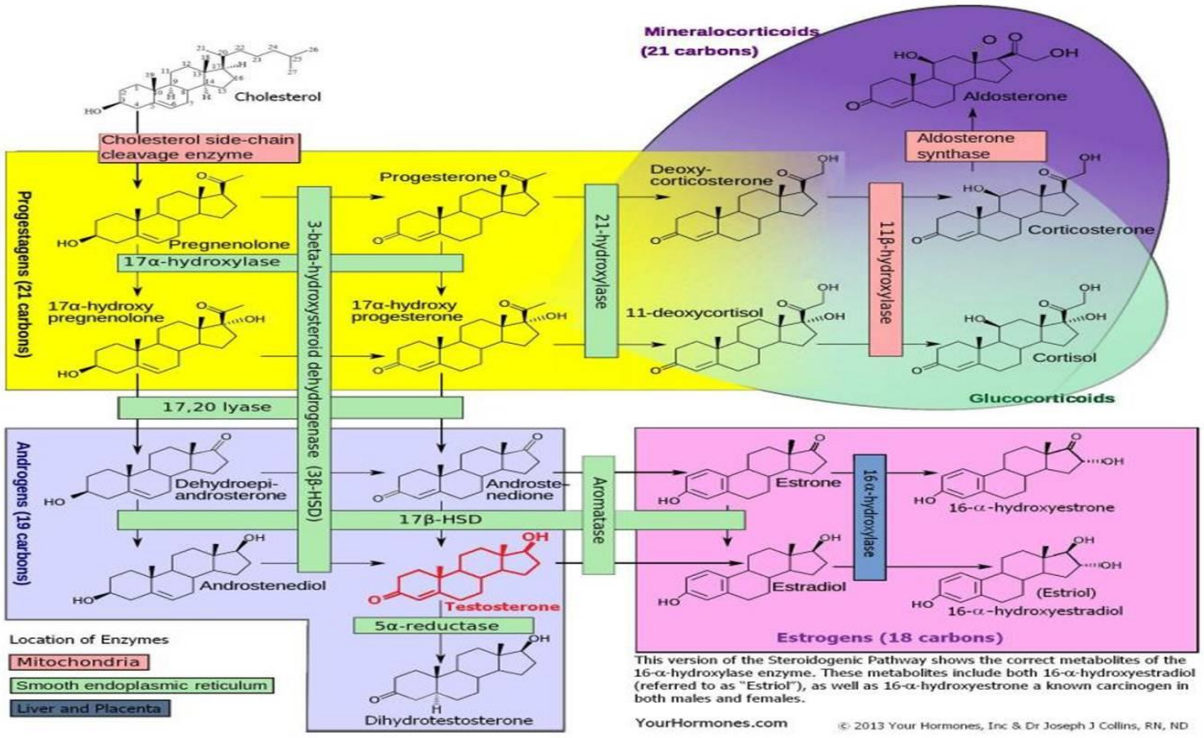
and pinealectomy stimulated cancer tumorigenesis (Mhatre et al., 1984; Shah et al., 1984; Tamarkin et al., 1981). Exogenous melatonin administration exerts anti-initiating and oncostatic activity (Anisimov et al., 1999; Mocchegiani et al., 1999; Musatov et al., 1999) in various chemically induced cancers. More recent studies demonstrated that chronically advancing the phasing of light exposure is associated with malignant progression in tumor-bearing mice (Filipski et al., 2004) . Furthermore the anti-cancer effects of melatonin depend on the circadian stage, as suggested by a number of animal studies (Bartsch & Bartsch, 2006). Therefore repeated changes of the timing of the light/dark cycle and melatonin phase, as experienced by night shift workers, might be an important carcinogenic mechanism. A possible interplay between melatonin and sex hormones has been also suggested by the melatonin hypothesis, as an indirect oncostatic mechanism.

1.4.2.2 Sex hormones

The circadian timing system is closely related to the endocrine system. A functional master clock located in the suprachiasmatic nuclei (SCN) of the hypothalamus is necessary for rhythmic steroid synthesis and excretion (Karatsoreos & Silver, 2007; Ota et al., 2012). It has been hypothesized that exposure to light at wrong times, such as experienced during night shift work, can disrupt normal melatonin synthesis which in turn may increase estrogen production (Stevens, 1987). Although melatonin has potential anti-estrogenic effects (Alvarez-Garcia et al., 2013; Cos et al., 2005; Srinivasan et al., 2008), an inverse association between endogenous melatonin and estrogens has not yet been confirmed in humans (Graham et al., 2001; Langley et al., 2012; Nagata et al., 2008; Schernhammer et al., 2006a). Some observational studies reported higher plasma estrogen levels related to long-term exposure to night shift work in women (Nagata et al., 2008; Schernhammer et al., 2004), while others found no changes in estrogens, androgens and progestagen levels after night shift work. A small number of sex hormones have been evaluated, mostly estrogens, while disruption of androgens and progestagens or their metabolites has not been sufficiently evaluated.

The possible effect of night shift work on estrogen, androgen and progestagen synthesis and metabolism (Figure 1.4.2.3) is largely under investigated, especially in men. Possible disruption of sex hormones due to night shift work and circadian disruption might explain in part the increased risk of breast and prostate cancer observed among night shift workers.

Figure 1.4.2.3 Simplified overview of steroid synthesis and metabolism (Credit:YourHormones.com)



1.4.3 Sleep disruption

Acute sleep restriction and long-term sleep deprivation are common among night shift workers (Sallinen & Kecklund, 2010). A meta-analysis of 36 studies showed that night shift work is associated with reduced sleep length (Pilcher et al., 2000). In the long term sleep deprivation and has been associated with chronic diseases including cardiovascular disease, diabetes and some cancers (Blask, 2009; Faraut et al., 2012). Sleep duration has been hypothesized to be associated with breast, prostate and colorectal cancer risk with inconclusive results (Lu et al., 2013). Sleep duration determines the length of light exposure and may as well influence melatonin levels. Serum melatonin concentrations have shown to be lower in habitual short sleepers (less than 6 hours) than in long sleepers (more than 9 hours). Sleep deprivation is found to have a profound effect on the endocrine function and hormones such as melatonin, cortisol, prolactine and sex hormones (Blask, 2009). Furthermore sleep disruption may increase cancer risk by affecting immune function and circadian regulation. In laboratory studies insufficient sleep has been associated with deregulation of genes expression and with metabolomic changes (Davies et al., 2014; Moller-Levet et al., 2013). Genes affected by insufficient sleep were associated with circadian rhythms (PER1, PER2, PER3, CRY2, CLOCK, NR1D1, NR1D2, RORA, DEC1, CSNK1E), sleep homeostasis (IL6, STAT3, KCNV2, CAMK2D), oxidative stress (PRDX2, PRDX5), and metabolism (SLC2A3, SLC2A5, GHRL, ABCA1). Short term sleep disruption has safety implications in night shift workers through increased sleepiness and propensity to accidents at work and over commuting (Sallinen & Kecklund, 2010). Overall findings suggest that sleep-related factors may raise the possibility of mechanisms beyond suppressed melatonin levels to explain the relation between shift-work and breast cancer.

2. RATIONALE

Recent experimental evidence suggests that night shift work and increasing use of electrical light at night might be associated with increased risk for cancer. Most of the human evidence has focused on female breast cancer and evidence on other tumors, such as prostate cancer is scarce. Epidemiological studies evaluating night shift work and breast cancer have shown some inconsistency in results but also shift work definitions, and often lacked important exposure information such as duration or intensity of night shift work. More studies evaluating this hypothesis are thus needed, with detailed shift work information. Furthermore chronotype is an individual characteristic that describes circadian phase and correlates with the personal preference for activity in the morning or evening. It has been suggested that chronotype relates to shift work adaptation and possibly modifies the association between night shift work and cancer. This is a new hypothesis that has been largely understudied and might help identify susceptible population groups.

Mechanisms underlying the association between night shift work and cancer risk remain unclear. Light induced melatonin suppression experienced by night shift workers is one of the main hypotheses that might explain the observed increased risk for cancer; however this has not yet been confirmed in populations of night shift workers. Disruption of sex steroid hormones, possibly mediated by melatonin, might provide another biologically plausible explanation for the increased risk observed among night workers particularly for hormone-dependent tumors. However existing biomarkers studies in humans have focused on female night shift workers, examined a small number of sex hormones, and have not taken into account the circadian variability of some of these hormones. Studies evaluating the 24-h variation of melatonin and sex steroid hormones are needed, including also male night workers, in order to elucidate the role of circadian and endocrine

disruption as possible underlying mechanisms in the causal pathway between night shift work and cancer risk.

3. OBJECTIVES

The main objective of this thesis was to evaluate the association between night shift work and breast and prostate cancer risk and identify possible underlying mechanisms for this association. To address this main aim the following specific objectives were developed:

- To evaluate if permanent and rotating night shift work was associated to prostate cancer risk in men in the MCC-Spain study, taking into account individual chronoytpe and disease severity (Paper I).

- To evaluate the association between permanent and rotating night shift work and breast cancer risk in women in the MCC-Spain case-control study (Paper II).

- To evaluate changes in the 24-h melatonin rhythm in male and female night shift workers compared to day workers (Paper III).

- To evaluate changes in the 24-h rhythm of sex steroid hormones and metabolites in current night and day workers and evaluate the possible interrelation between melatonin and sex hormones (Paper IV).

Additional work has been conducted related to the main objectives of the thesis and includes the evaluation of circadian disruption and colorectal cancer risk, the effect of sleep disregulation and disruption on colorectal, prostate and breast cancers and the evaluation of main genetic effects and gene-environmetn interactions for genes in circadian, melatonin and sleep related pathways. This work is not presented as part of this thesis but has been part of the work the candidate conducted during the last few years.

4. METHODS

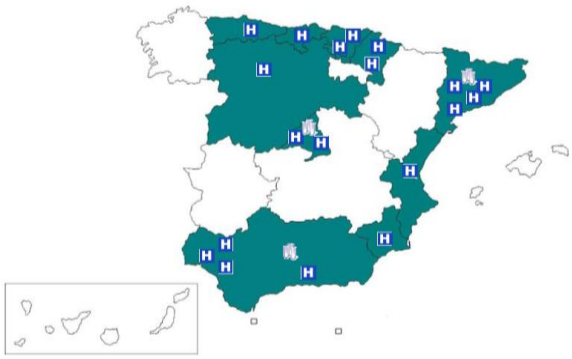
This chapter provides an overview of the methods used in the two studies that this thesis was based on. The study design, study population, inclusion criteria and other materials is described for both studies. A more specific and detailed description of the studies can be also found in the papers included in the next chapter.

4.1 The Multi-Case-Control (MCC)-Spain study

Study design

The MCC-Spain study is a population based multi-case control study in Spain, evaluating environmental and genetic factors associated with frequent tumors (breast, prostate, colorectal, gastric, chronic lymphocytic leukemia) in men and women. In total 20 hospitals in 11 autonomic communities (Asturias, Barcelona, Cantabria, Granada, Guipuzcoa, Huelva, Leon, Madrid, Murcia, Navarra, Valencia) in Spain (Figure 4.1).

Figure 4.1 Participating centers and hospitals in the MCC-Spain study highlighted in green.



Study population

Recruitment of cases and controls took place from 2008 through 2014. All incident cancer cases diagnosed in the participating hospitals, with a histological confirmation of the diagnosis, were invited to participate in the study. A common set of population

controls were selected for all tumors randomly from the rosters of General Practitioners at the Primary Health Centers (PHC). They were contacted on behalf of their doctor and invited to participate to the study. Both cases and controls had to be living in the catchment area of the hospitals for at least 6 months prior to diagnosis or interview. Controls were frequency matched by sex, age and recruitment centre. Subjects incapable to participate in the interview due to communication difficulties or excess impairment of physical ability were excluded.

We included 1708 breast cancer cases, 1778 female controls, 1095 prostate cancer cases and 1338 male controls with shift work information. Response rates were on average 72% among cases and 53%, ranging from 30-77% among centers, among controls with valid telephone numbers in the PHC rosters.

Data collection

Information was collected through personal interviews with the participants performed by trained personnel. Data was collected on socio-demographic factors, lifestyle habits, residential and occupational history, reproduction, drug use, personal and family medical history. Diet habits were assessed using a self-administered food-frequency questionnaire.

Night shift work was assessed through lifetime occupational history. Specific questions were included on shift type (day, night, rotating), shift schedule (start time, end time), hours of work, night shifts worked/month, year of beginning and end of each job. A common night shift work definition was used and permanent and rotating schedules were assessed separetely.

Chronotype and details on time schedules for rotating night workers were assessed through a telephone interview in a follow-up in 2013. We used the two-page validated Munich Chronotype Questionnaire (MCTQ) and we caclulated the mid sleep time on a free day

corrected for oversleep. We categorized chronotype using 7 (extremely morning, moderately morning, slightly morning, neither, slightly evening, moderately evening, definetely evening) , 5 (extreme/moderately morning, slightly morning, neither, slightly evening, moderately/extremely evening) and 3 categories (morning, neither, evening) in the statistical analysis.

Statistical analysis

We calculated crude and adjusted Odds Ratios (OR) with 95% confidence intervals (CI) for having ever done shift work and night shift work, using different metrics including types of night work and lifetime cumulative exposure (duration and frequency). We assessed the direct association of chronotype and cancer risk and evaluated chronotype as an effect modifier for the night shift work–cancer association. We tested possible interactions between night shift work and chronotype, obesity. We also analyzed night shift work by different clinical/pathological stages of prostate and breast cancer using polytomous logistic regression models. In order to increase efficiency and minimize selection bias, we performed multiple imputation of missing values using chained equations.

4.2 The circadian biomarkers study

Study population

This was a cross-sectional study including permanent night and day workers of both sexes in four companies (2 hospitals, car industry and a train company) recruited from March to June 2011 in Barcelona and surroundings. Subjects were not eligible for participation if they had personal cancer history, were taking oral contraceptives or hormonal therapy or had been pregnant 6 months prior to the study. 75 night and 42 day workers of both sexes, aged 22-64 years were recruited in the study.

Data collection

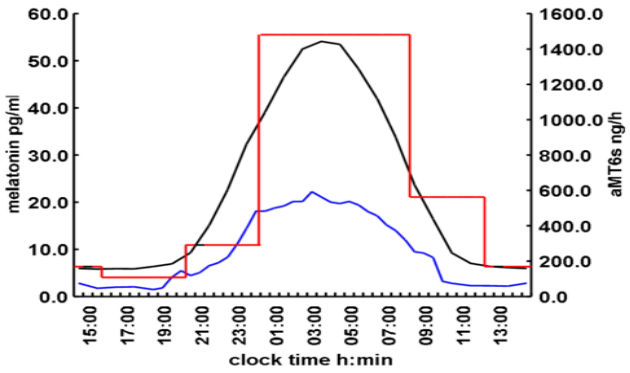
We collected information on sociodemographics, lifestyle habits, night shift history, sleep habits, physical activity, smoking, alcohol and caffeine consumption, reproduction, health symptoms and medication through a personal interview. Occupation information included start and stop times, number of shifts per week, main tasks and activities and years worked at night. Diurnal preference was assessed using the Morningness-Eveningness Questionaire (Horne & Ostberg, 1976).

Hormone measurements

Participants were asked to collect one urine sample from each natural void over a 24-h working day or night and store them in the fridge. Samples were transported on ice and were frozen at -80 ºC until analysis. We measured urinary concentrations of 6-sulfatoxymelatonin (aMT6s), the principal metabolite of melatonin, in a total of 1030 samples at the Chronobiology Group, University of Surrey, UK, using a radioimmunoassay (Stockgrand, Ltd., Guildford, Surrey, UK). The aMT6s analyses were performed by myself as part of a three and a half month training I did as part of my thesis in the Chronobiology group in collaboration with Dr. Debra Skene and the University of Surrey. The concentrations in

urine (red line) correlate very well with plasma (black) and saliva (blue) concentrations (Figure 4.2.1).

Figure 4.2.1 Agreement between urine 6-sulfatoxymelatonin (red line) and plasma (black line) and saliva (blue line) melatonin



Concentrations of steroid hormones and metabolites including estrogens, progestagens, androgens and corisol (Figure 4.2.2) were measured in a total of 899 urine samples, using gas chromatography and mass spectrometry (GC-MS). Analyses were performed by the Bioanalysis Research Group at IMIM, in Barcelona, Spain.

Figure 4.2.2 List of steroid hormones and metabolites measured in the study

3 Estrogens	**10 Androgens**		**3 Cortisol**
estradiol	testosterone	11β-OH-androsterone	cortisol
estone	epitestosterone	4-androstenedione	cortisone
estriol	DHEA	6α-OH-androstenedione	5β-tetrahydro cortisol
3 Progestagens	androsterone	3α,5α-androstanediol	
pregnanediol	etiocholanolone	3α,5β-androstanediol	
pregnanetriol			
16-androstenol			

Creatinine levels were determined in all urine samples by the same laboratory using the manual picric acid, sodium hydroxide colorimetric method (Randox Laboratories Ltd.) in order to account

for dilution variability and duration between consecutive samples. All hormone concentrations are expressed in ng/mg creatinine.

Light exposure measurements

Personal light measurements

Participants wore a light intensity data logger (HOBOware, Onset Computer Corporation) that continuously (every 12 or 15 seconds) recorded their ambient light exposure over an approximate 24 hour period, simultaneously with the urine collections. The logger was relatively small in size (5.8 x 3.3 x 2.3 cm) and light in weight (18 g) and was carried at the shoulder level (Figure 4.2.3). This position was used to obtain measurements that would approximate the amount of light reaching the retina.

Figure 4.2.3 HOBO data logger used for personal light monitoring placed at the shoulder level.

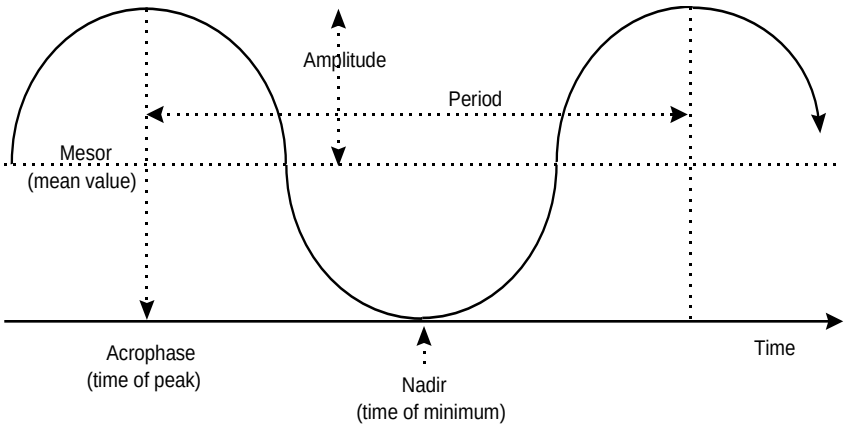
Ambient light measurements

Ambient light measurements were also performed using a radiometer DELTA OHM, model HD24402 including both intensity (lux) and irradiance **(W/m^2)** measurements in collaboration with the labour department of the local government**.**

Statistical analysis

Individual cosinor analysis was used to evaluate the rhythm of 6-sulfatoxymelatonin, individual and sex hormone metabolites and total estrogen, progestagen and androgen production. Cosinor analysis is a curve fitting procedure used in the analysis of rhythms with a cyclic nature and an approximate 24-h period (Mikulich et al., 2003). Metabolites´s acrophase (peak time), mesor (24-h mean) were derived for each subject (Figure 4.2.4) and compared between night and day workers. Cosinor is the only rhythm analysis method in common use which gives a value for all these features.

Figure 4.2.4 Cosinor curve and derived cosine parameters: mesor (24-h mean), amplitude (maximum-mean), and acrophase (peak time).



5. RESULTS

5.1 Paper I: Night shift work, chronotype and prostate cancer risk in the MCC-Spain case-control study

5.2 Paper II: Breast cancer risk and night shift work in a case-control study in a Spanish population: the MCC-Spain study.

5.3 Paper III: Circadian variation of melatonin, light exposure and diurnal preference in day and night shift workers of both sexes

5.4 Paper IV: Increased sex hormone production in male and female night shift workers

5.5 Commentary: Shift work and breast cancer: do we need more evidence and what should this be?

5.2 Paper I

Night shift work, chronotype and prostate cancer risk in the MCC-Spain case-control study

Kyriaki Papantoniou, Gemma Castaño-Vinyals, Ana Espinosa, Nuria Aragonés, Beatriz Pérez-Gómez, Javier Burgos, Inés Gómez-Acebo, Javier Llorca, Rosana Peiró, Jose Juan Jimenez-Moleón, Francisco Arredondo, Adonina Tardón, Marina Pollan, Manolis Kogevinas

Under review in the International Journal of Cancer

Papantoniou K, Castaño-Vinyals G, Espinosa A, Aragonés N, Pérez-Gómez B, Burgos J, Gómez-Acebo I, Llorca J, Peiró R, Jimenez-Moleón JJ, Arredondo F, Tardón A, Pollan M, Kogevinas M. Night shift work, chronotype and prostate cancer risk in the MCC-Spain case-control study. Int J Cancer. 2015 Sep 1;137(5):1147-57. doi: 10.1002/ijc.29400

5.1 Paper II

Breast cancer risk and night shift work in a case-control study in a Spanish population: the MCC-Spain study.

Kyriaki Papantoniou, Gemma Castaño-Vinyals, Ana Espinosa, Nuria Aragonés, Beatriz Pérez-Gómez, Eva Ardanaz, Jone M Altzibar, Vicente Martin Sanchez, Inés Gómez-Acebo, Javier Llorca, David Muñoz, Adonina Tardón, Rosana Peiró, Rafael Marcos-Gragera, Marina Pollan, Manolis Kogevinas

To be submitted

Papantoniou K, Castaño-Vinyals G, Espinosa A, Aragonés N, Pérez-Gómez B, Ardanaz E, Altzibar JM, Sanchez VM, Gómez-Acebo I, Llorca J, Muñoz D, Tardón A, Peiró R, Marcos-Gragera R, Pollan M, Kogevinas M. Breast cancer risk and night shift work in a case-control study in a Spanish population. Eur J Epidemiol. 2015 Jul 24. doi:10. 1007/ s10654-015-0073-y

5.3 Paper III

Circadian Variation of Melatonin, Light Exposure, and Diurnal Preference in Day and Night Shift Workers

Kyriaki Papantoniou, Oscar J. Pozo, Ana Espinosa, Josep Marcos, Gemma Castaño-Vinyals, Xavier Basagaña, Ferran Calduch Ribas, Mirabent Joan, Jordi Martín, Gemma Carenys, Celia Reyes Martín, Benita Middleton, Debra J. Skene and Manolis Kogevinas

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5.3 Paper IV

Increased sex hormone production in male and female night shift workers

Kyriaki Papantoniou, Oscar J. Pozo, Ana Espinosa, Josep Marcos, Gemma Castaño-Vinyals, Xavier Basagaña, Elena Juanola Pagès, Joan Mirabent, Jordi Martín, Patricia Such Faro Amparo, Gascó Aparici, Benita Middleton, Debra J. Skene and Manolis Kogevinas

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5.5 Commentary

Shift work and breast cancer: do we need more evidence and what should this be?

Kyriaki Papantoniou and Manolis Kogevinas

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Papantoniou K, Kogevinas M. Shift work and breast cancer: do we need more evidence and what should this be? Occup Environ Med. 2013 Dec;70(12):825-6. doi: 10.1136/oemed-2013-101630

6. DISCUSSION

This chapter is complementary to the discussion of the papers and aims to broader discuss and interpret the main findings of this thesis in the context of the existing literature providing perspectives on future research.

6.1 Contribution to the current knowledge

When the IARC evaluated the carcinogenicity of shift work epidemiologic studies were limited in number, though findings seemed fairly consistent, at least for breast cancer risk (IARC, 2010; Straif et al., 2007) (Monograph 2007, Megdal 2005). However new studies published after the IARC Monograph provide a more complex picture of the association between night shift and breast cancer and underline the need for better exposure assessment and extensive evaluation of confounders and effect modifiers.

6.1.1 Night shift work and cancer risk

Our findings provide more epidemiological evidence on the association between night shift work and cancer risk. Paper I and II suggest that night shift work might be associated with an increased risk for the two most common cancers, breast and prostate cancer, at least for some exposure groups. We assessed all 3 characteristics suggested by the IARC consensus group: type, duration and intensity (Stevens et al., 2010). Only one previous study has included this potentially relevant information in their assessment for breast cancer (Hansen & Lassen, 2012) while no previous study has done so for prostate cancer risk. Cumulative years of exposure as well cumulative frequency which took into account the number of shifts worked per month. Duration of shift work not taking into account the number of such shifts (e.g. nights per week), is a poor proxy for dose. We found higher risks not only for subjects that had been engaged to night shift work for the longest periods but also those that had more intense schedules in combination with longer

durations of exposure. However this was mostly true for the permanent night shift schedules. Rotating night shift work was associated with breast cancer risk also after short exposures (<5 years). These findings are partly in line with some studies that showed increased risks after prolonged periods of exposure when focusing on a single type of occupation, for example nurses. We did not find significant differences between the permanent and rotating night shift schedules in terms of breast and prostate cancer risks, although we found an indication of a differential pattern of cumulative risk between the two shift schedules. This might partly explain the lack of dose-response association in other studies, where a variety of occupations was evaluated and the type of night shift work was unknown.

A novel finding of this thesis is the possible contribution of night shift work in cancer progression reflected in higher risks for prostate cancer tumors with worse prognosis (Paper I). None of the previous studies that observed an association bewtween night shift work and prostate cancer risk reported on disease prognosis (Conlon et al., 2007; Kubo et al., 2011; Kubo et al., 2006; Parent et al., 2013; Russo & Russo, 2008). One study observed an association between current shift work and elevated prostate-specific antigen (PSA) levels, that might be predictive of prostate cancer risk and worse prognosis (Flynn-Evans et al., 2013). Furthermore a recently published study found an inverse association between melatonin levels and advanced prostate cancer (Sigurdardottir et al., 2014). One conference report has provided some evidence for decreased survival of breast cancer among both fixed and rotating female night shift workers (Hansen, 2014). Circadian disruption accelerates tumor development and growth in experimental animal models. Night shift work that involves circadian disruption may be associated with more advanced stages of cancer and decreased survival. Given the general lack of knowledge on factors associated with worse prognosis and survival the replication of this finding in future studies might be valuable.

Furthermore our results support the hypothesis that night work plays a role in breast cancer, particularly in women who started working at night before first full-term pregnancy, a period where mammary gland cells are incompletely differentiated and possibly more susceptible to circadian disruption effects (Paper II). One previous study observed results similar to ours (Menegaux et al., 2013). Although measuring environmental exposures during critical periods of breast development in a woman's life is a key issue for identifying exposures that may lead to breast cancer (Russo & Russo, 2008), the role of night work during these critical exposure windows has not been specifically investigated in epidemiological studies.

Individual characteristics might be crucial in identifying groups with higher susceptibility to circadian disruption. This is a largely understudied area of research and evidence on breast cancer is scarce while there is no data on prostate cancer (Hansen & Lassen, 2012). We provided evidence that chronotype may modify the association between night shift work and cancer risk. However, our findings are not in line with the initial predictions that morning types being more susceptible to night shift work related circadian disruption would present a higher cancer risk (Erren, 2013). We found higher overall breast and prostate cancer risk among night shift workers with an evening preference (Paper I & II). A two-fold increase in breast cancer risk was also observed among night shift workers with a morning chronotype and short exposures, based on small numbers (Paper II). It is likely that morning types drop out of night shift work, due to coping difficulties, while evening types remain for longer periods when risk is observed. However adjustment for years of night shift work did not change the results. The initial hypothesis was based on the fact that chronotype is associated to shift work adaptability (Saksvik et al., 2011). Night shift workers with an evening chronotype may adapt easier or faster to their shift schedule, compared to morning types (Arendt,

2012), by shifting their circadian rhythms. However although adaptation is beneficial in the short term, by reducing night sleepiness at work and improving daytime sleep, not much is known for its long term health implications and less on its effect on future cancer risk. Phase shifts of the circadian system are detrimental for experimental animals. In humans a higher number of consecutive night shifts has been associated with a higher breast cancer risk (Lie et al., 2011). Therefore the shifting of the circadian phase, as occurs with adaptation after several consecutive night shifts, might be risky, at least for some health outcomes.

6.1.2 Exposure assessment for night shift work

One of the main limitations of the existing literature is the crude exposure assessment in some studies and the inconsistent definitions for night shift work (Bonde et al., 2012; Ijaz et al., 2013; Jia et al., 2013). The lack of uniformity in definition of shift work is a crucial issue for the interpretation and comparison of the results of this thesis with previous studies. Some studies focused on rotating shifts, others on graveyard shifts (beginning work after 7p.m. and ending before 9a.m.) and others on night shift. Each study has defined night shift as work that takes place entirely or partly from as early as 18:00 in the afternoon up to as late as 09:00 in the morning. In the MCC-Spain study night shift work assessment was based on the exact time schedules (from 00:00 to 06:00), and the % spent in each shift. A common definition was applied for the night period, and subjects were subsequently reclassified so as to fit the predefined categories. However a self-reported shift evaluation of each job (day, night or rotating) was also available. Analysis was also performed using the self-reported classification and results were slightly different, however not in a given direction, indicating non-differential exposure misclassification. The inconsistencies in definitions of night shift work observed in epidemiological studies were also reflected in subjects' evaluations of jobs, e.g. classifying jobs with start time at 03:00 o´clock as a day job. Therefore, self-reports for night shift work might not be as reliable for use in future studies.

Underestimation of effect estimates in particular for breast and prostate cancer risk (Papers I and II) is possible due to the lack of defining an adequate comparison group of completely unexposed subjects. Night shift work is a proxy for light at night exposure and sleep deprivation. Almost all subjects are exposed to light at night and especially in a ¨late¨ society such as the Spanish society. It has become very common to adopt activities such as television viewing, or computer and mobile phone use before bedtime, and therefore ultimately everyone is exposed to light at night and possibly

experience some degree of circadian disruption without being engaged to night shift work. Furthermore subjects that work in day schedules might also experience sleep deprivation and circadian disruption, particularly extreme late types with early morning shifts (Erren, 2013).

6.1.3 Mechanisms: hormonal changes

In evaluating causality of an association it is important to examine biologic plausibility of the associations and describing and understanding underlying mechanisms. The protective role of the hormone melatonin as an oncostatic agent has been long discussed (Blask et al., 2005; Mediavilla et al., 2010). However, the disruption of melatonin after night shift work has not been consistently reported in field studies with real-life night shift workers . On the other hand disruption of sex steroids hormones, possibly mediated in part by melatonin disruption, might be involved in carcinogenic processes for endocrine related tumors, such as breast and prostate cancer.

The findings of the present thesis adds to the existing knowledge gap regarding hormonal changes in night shift workers (Paper III and IV). We reported changes not only in melatonin but also in a wide range of sex steroids in both sexes. According to one of the first proposed mechanisms, known as the "melatonin hypothesis", night shift work was associated to breast cancer through light induced melatonin suppression and subsequent estrogen increase (Stevens, 1987). Although this hypothesis has been widely cited it is not yet confirmed in humans. Night shift work was associated with lower melatonin levels among night workers, compared to day workers, even under low light levels (Paper III). We also found an increase in androgens and progestagens, that was independent of melatonin production (Paper IV). Although our findings are in line with the melatonin hypothesis : decrease in melatonin and increase in sex hormones, we did not find evidence for an association between urinary melatonin and estrogens or other sex hormones in either day or night workers. Our findings point to a direct effect of night shift work, sleep, light at night or circadian disruption on sex steroid production, in addition to melatonin supression.We cannot determine whether the hormonal changes described represent acute effects or whether there is a more chronic up-regulation of the hypothalamus–pituitary–gonadal (HPG) system, or both. Higher

gonadotropin levels (LH and FSH) have been reported among permanent night workers (Davis et al., 2012). There is some evidence that light might dysrupt sex steroid hormone production through an increase of LH and FSH, although more evidence is needed (Kripke et al., 2010).

Melatonin is the the best biomarker of circadian phase, with a well established 24 hour rhythm. Most previous studies with real life night workers were unable to assess rhythmicity of melatonin due to the fact that the assessment was based on spot samples or a few samples during the day . Rhythms of sex steroids are not well-studied and there is some evidence of diurnal variation with peak production obseved in the morning. No previous study in night shift workers took into account this possibility and thus results were confounded by circadian stage. We were able to assess 24-h rhtythms of melatonin and 16 steroid hormones and metabolites in shift workers in the field. We used a type of rhythm analysis often used in experimental studies, the cosinor analysis, to estimate the peak time of production for each hormone. We found a later peak time of melatonin, as expected, in night compared to day workers and evidence of adaptation among subjects with more consecutive night shifts (Paper III). We also found evidence of disrupted rhythmicity in sex steroids with a delay of androgens among night workers (Paper IV). Given that steroidogenesis is under circadian control, circadian disruption might have an effect on the timing of the production of the sex steroid hormones (Karatsoreos et al., 2007; Ota et al., 2012). However this study lacked baseline measurements, thus, the degree of circadian disruption/adaptation is unknown within individuals. We partly accounted for that by adjusting for chronotype that is a measure of circadian phase. The disruption of the rhythm might be an additional mechanism for the increased risk for cancer. Animal studies show that the oncostatic effects of melatonin were diminished if production took place over the wrong biologic period (Bartsch & Bartsch, 2006). Therefore in adapted night shift workers with a phase shift of their melatonin

production daily levels may not change, but the protective effect of melatonin might be lost. The consequences of a mistimed sex hormone production on the mammary and prostate gland, have not been studied yet. In this study, we also evaluated chronotype and found a higher supression of melatonin among night shift workers with a morning preference (Paper III). This might be explained by the fact that morning types take longer to phase shift their melatonin rhythms, therefore may experience a larger melatonin suppression after light at night exposure. On the other hand evening types might maintain 24-h melatonin levels but melatonin production might take place at the wrong chronobiological time and the protective-effects of melatonin might be lost.

Sleep is closely related to the circadian system although it is not sufficient to synchronize it. Sleep controls light exposure of the retinae through the opening and closing of the eyes. Night shift work can affect the circadian rhythmicity as well as disrupt sleep (Pilcher et al., 2000; Sallinen & Kecklund, 2010). Some sex hormones such as testosterone can also be strongly influenced by sleep, particularly its timing and duration (Axelsson et al., 2005). Since night shift work is closely linked to daytime sleep, it is possible that some of the observed effects on androgen production are also due in part to acute sleep restriction. It is difficult to disentangle the effects on hormone production of sleep, circadian disruption and light exposure, especially in field studies. It is not clear whether circadian or sleep disruption have the strongest influence on acute or long term adverse health effects and if there are interactions. Both in the MCC-Spain study and the biomarkers' study we evaluated sleep habits. We considered variables describing sleep duration, quality and timing in all the analysis as possible confounding factors, and were included in the final models were appropriate. We found no evidence of interaction between sleep and circadian disruption, although sleep problems were quite often reported. In a separate analysis, not included in this thesis, we are evaluating sleep duration, quality and timing as independent risk

factors for different types of cancer (EPICOH conference 2014). Future research should attempt to disentangle the relative roles of circadian, sleep disruption and light exposure on health effects, as this might be very helpful for preventive actions.

6.2 Methodological issues: strengths and limitations

6.2.1 Study design

The use of data from the multi case-control study (MCC)-Spain study was one of the main strengths of this thesis. The case-control design was appropriate for the evaluation of cancer that is a disease with a long induction period. Furthermore this study design allows multiple collection of information related to exposure (night shift work) and other occupational and environmental factors retrospectively with a fair amount of details. Case-control studies have some limitations that will discussed in continuation including controls' selection bias, exposure misclassification and recall bias. For the biomarkers' study we used the cross-sectional design or semi-experimental and on the basis of the exposure we included subjects employed in night shift work and day workers from the same work environments as controls. In both case-control and cross-sectional studies temporality is an issue since measurement of exposure and outcome occurs at the same point in time. For cancer the induction period is long and might last for 10 or more years and therefore the lag-time needs to be taken into account in this type of analyses (Hankinson, 2008; Lorelei A Mucci, 2008).

6.2.2 Statistical power

Power is a consideration for all studies but paricularly when stratified analysis relevant to study hypothesis are to be performed. The levels of power were calculated before analysis for different sample sizes for n1 cases and n2 controls (N=n1+n2). In order to proceed to this calculations it was assumed that the prevalence of exposure was 20%, that the background OR estimation was 1.5 (Megdal et al., 2005) and that a two-sided test at 5% level of significance would be used with a case-control ratio of 1:1. Including 1200 subjects, we had 80% power to detect the associations. We finallly had a larger population size than what

initially expected (1095 prostate cancer, 1708 breast cancer cases and 3116 population controls). However the magnitude of the effects were generally smaller than the one assumed in our initial power calculations and many of the estimates we reported were not statistically significant. The percentage of night shift workers was also rather low, in the MCC Spain stduy particularly among women. Therefore although the MCC-spain is large in size, numbers in stratified analysis were small and therefore risk estimates less precise. That might also explain why we found no evidence of effect modification by most of the hypothesized factors (Paper I: age of diagnosis, BMI, education, smoking; Paper II: menopausal status, parity, chronotype: Paper III: chronotype, menopausal status). Other case-control studies with similar exposure assessment and number of recruited subjects also did not find statistically significant or borderline significant associations and limited power has been suggested as a possible explanation (Fritschi et al., 2013; Grundy et al., 2013; Menegaux et al., 2013; Pesch et al., 2010). A pooled analysis of published studies with similar protocols, as discussed in Paper V, might be a useful approach, to overcome power problems.

6.2.3 Confounders and effect modifiers

All findings reported in this thesis were based on observational studies, and numerous factors may confound the described associations in real life settings. Confounding is generally considered as a potential important factor in shift-work research, due to the possible differences between day an night shift workers, and was one of the reasons why the IARC working group was skeptic about the epidemiological findings at the time of the evaluation. As frequently happens in epidemiological studies control for confounding tends not to be systematic across studies, also because patterns of exposure and co-exposure differ between societies. It is also noteworthy and surprising that several previous studies did not identify major confounding by factors such as age, socioeconomic status, number of children, BMI, alcohol and

smoking. On the other hand, a number of studies have adjusted for a large number of variables without justifying their selection. In case-control studies controlling for variables that are unrelated to the outcome may result to overadjustment bias. We selected confounders using Direct Acyclic Graphs (DAGs) in combination with classical statistical criteria (change-in-estimate) (Evans et al., 2012; Weng et al., 2009). DAGs provide a visual picture of the known and suspected associations between exposure, measured and unmeasured covariates and outcome. DAGs help identify possible sources of confounding and therefore provide an important tool in the building of etiologic models for epidemiological studies. Although the use of DAGs is becoming common in epidemiologic studies, their use in previous research on the night shift work cancer association has been limited and only a systematic review, has made use of this tool (Ijaz et al., 2013). It has been hypothesized that lifestyle and sociodemographic characteristics may vary considerably between day and night workers and confound the results significantly. It is unknown, however, whether subjects with a less healthy lifestyle (smoking habits, diet, coffee consumption, sleep problems) are selected into night shift work or if night shift workers adopt a different lifestyle because of their working schedule. In our data, the two groups (night shift workers vs day workers) did not differ on most variables like age, body mass index (BMI) and parity and in most analysis adjusted models did not provide very different estimates than the unadjusted ones. We also performed multiple imputations in order to retrieve missing information for all possible confounders. Still we did not find much evidence for strong confounding although we examined awide range of potentially confounding factors in both studies (MCC-Spain study and Biomarkers study). However adjustment for confounding is important, and analysis on confounders, mediators and effect modifiers needs to be carefully done in future studies.

A strength of this thesis was the evaluation of chronotype in both studies. The possible role of chronotype as an effect modifier of the

night shift work-cancer association is a new and tempting hypothesis. However evidence is still insufficient and how much these factors may modify the desctribed associations remains to be seen and clearly we need more data. In the biomarkers study we used the Morningness-Eveningness Questionnaire (MEQ) for the assessment of diurnal preference (Horne & Ostberg, 1976). The MEQ, used in most circadian association studies so far, is an excellent instrument that has been used extensively in research. However, the questions of the MEQ are mostly subjective, assessing time-of-day preferences based on a personal "feeling best rhythm" or on hypothetical situations (e.g., "approximately what time would you get up if you were entirely free to plan your day?"). The MEQ does not assess actual times (e.g., of sleep or activity) and does not distinguish between free and work days. The use of the MCTQ in the MCC-Spain study, however, allowed a quantitative assessment of entrained phase (chronotype) by taking all the considerations mentioned above into account. Furthermore the use of the MCTQ was a methodological improvement compared to previous studies that had used a single question of the original version of the MCTQ. However, the MCTQ shows good correlation with both the MEQ score and the single MCTQ question (Roenneberg et al., 2003; Zavada et al., 2005).

6.2.4 Bias and generalizability of results

This analysis has limitations inherent to all case-control study designs (Paper I and II). Interview based case-control studies like the present are normally prone to information bias. The retrospective estimations of exposures based on self reported occupational history may lead to exposure misclassification. However, differential recall between cases and controls for night shift work is unlikely, since the night work-cancer association has not still been discussed in spanish media. Interviewer bias was minimised as interviews were done by well trained personnel. Main analyses were also performed stratifying by interviewer and results were unchanged. Selection bias might occur if exposed controls are more likely to be selected than non-exposed individuals and vice-versa. In the MCC-Spain study control subjects engaged to night shift work may less frequently have answered the phone calls due to daytime sleep compared to day workers. To avoid this bias, phone calls were scheduled and performed in different times during the day. We also repeated analysis excluding current workers and results remained unchanged.

A main purpose of research is to extrapolate the findings in other settings than the ones studied and to the general population. However, one has to be careful as many of the findings may be specific to the study population. Many of the previous studies focused on a single occupation, nurses, and in that case data may not be applicable in other occupations were work tasks and education levels differ. Furthermore other carcinogens present in the specific work environment might confound the described associations. Moreover, there are probably different selection processes according to occupation and shift system. This means that results from a certain shift working population may not be fully applicable to other groups of shift workers. Taken together, one should be careful with generalizations in other occupations and cultures and understand what processes are important in certain settings. The present thesis includes results from different work

places and occupations (Paper I & II: all occupations, Paper III & Paper IV: hospitals, car industry plant, and railway company) and also on both sexes. It is a strength of this thesis that the generalizations are built upon findings in different shift systems in different occupations, which increases the possibility that these findings are universal.

6.3 Public health implications

The public health consequences of the evaluations included in this thesis are important, since shift work is a very common occupational exposure with about 20% of the workforce in Europe doing some non-normal work schedule. The current prediction is that night shift work will become even more common in the future. Furthermore it is unlikely that the exposures (e.g. LAN) and activities that are leading to de-synchronisation of the biological clock will disappear in the future. As such it is important to develop prevention programs accounting for individual susceptibility and strategies to mitigate the potential risks, that can apply both for night shift workers as well as to the general public.

Shift work planning is important, although no shift work that involves night work is optimal. However coping strategies, including sleep assistance, light exposure regulation and chronobiology education might help night shift workers and their families. Night shift work before a first full-term pregnancy in a womans´s life needs to be avoided, especially for long durations. Long-term night shift work should be disencouranged for all workers and more intesive medical follow-ups might be needed in these workers. Finally individual characteristics, such as chronotype or diurnal preference might be useful in selection processes for night shift work.

With respect to cancer risk and other outcomes there is a controlversy on which type of night shift work (rotating or permanent night shifts) is preferable and also if adaptation is desirable or not. The only way to answer the controversy from a chronobiological point of view is to determine which is the more harmful: (a) working through one or two nights per week without changing the SCN phase (i.e., occasionally being in a state of external desynchronosis) or (b) changing SCN phase on a regular basis and thus, during the process of phase adjustment, suffering

from internal desynchronosis. Unfortunately, we currently do not know whether external desynchronosis or internal desynchronosis is the more harmful. Animal models are needed to tease out which of the two most adversely affects longevity. Until that model is available, decisions must rest upon the appropriateness of the task being performed (monotonous repetitive tasks are unlikely to fare well under rapid rotation) and the personal preferences of the workers involved.

There is currently an open discussion on whether night shift work should be included in national lists of occupational hazards that associate to cancer risk, for compensation purposes. Already in Denmark, it has been possible since 2007 to be compensated for breast cancer after long term (>20 years) night work. Although this is an open discussion more evidence and better understanding of the biologic mechanisms is probably needed before night shift work can be generally accepted as a human carcinogen. Actually, IARC has indicated that a re-evaluation of night shift work as a human carcinogen will be planned in the next five years (ref straif Lancet Oncology 2014).

The work done on circadian genetics is impressive and very promising. Genetic variations in clock genes may also affect adaptation and effects of circadian disruption on health. These are, however, understudied areas in population-based research and it might be too early to suggest the use of genetics as a tool of risk prediction or in planning of prevention policies in night shift workers.

6.4 Future research

This is a huge and quickly developing area of research including both epidemiological and experimental research. Clearly there is a need for multidisciplinary approaches and international cooperation. In this text I concentrate therefore on priorities for future research within the areas of research developed in CREAL.

We definitely need more studies on other tumor sites, such as prostate and colorectal cancer, to confirm the existing evidence on possible associations. A pooled analysis of all breast cancer studies is needed, applying common definitions for exposure, if this is feasible. We also need more studies that carefully evaluate different patterns of shift work, especially rotating shift work, with respect to cancer risk. Shift work rotation characteristics such as the speed and the direction of rotation, as well as the number of consecutive nights of work and days off may define groups of subjects with higher risk. In the MCC-Spain study this will be possible as detailed information on rotating shift schedules was collected in a follow up of all breast and prostate cancer cases and controls. The possible effect modification by chronotype, needs to be further explored through observational and experimental data. Chronotype need to also be evaluated with respect to cancer risk, as an independent risk factor. The influence of night shift-work and sleep patterns on survival of cancer has not yet been reported, though decreased longevity has been observed in experimental animals. We will follow-up patients of breast, prostate and colon cancer concerning death comparing non-day shift-workers with day-workers and take into account extent of disease at diagnosis.

Other sources of circadian disruption in the general population can be identified related to abnormal light-dark exposure, sleep patterns and diet habits. There is increasing evidence from experimental studies that alignment between central driven circadian rhythms (melatonin) and feeding time is important for metabolism and shifting patterns of food intake affects metabolic health, for

example obesity or insulin resistance.Within the MCC-Spain study the timing of different exposures wil be evaluated in addition to the dose or frequency of exposure. The timing of daily meals, sleep, physical activity and bedroom light exposure patterns will be evaluated with respect to breast and prostate cancer risk. Sleep disruption may also be a potentially modifiable risk factor for some cancers. Sleep duration, quality, and timing will be evaluated with respect to different tumours in the MCC-Spain study.

We also need more mechanistic studies, both experimental and semi-experimental field studies to better understand the underlying biologic mechanisms. Our results on sex steroid hormones provide a new and biological plausible hypothesis for a direct association between night shift work, circadian disruption and endocrine related tumors. These results should be replicated in larger studies. It will be also useful to try and disentangle the effects of night shift work, sleep disruption and light exposure with respect to hormonal changes and cancer risk in future studies. Furthermore there is a first indication that epigenetic mechanisms, such as circadian genes´ methylation patterns, may also be implicated in the association between night shift work and cancer risk and this is an emerging area for future research.

There is a strong and well described genetic component in circadian time –keeping system. Circadian genes polymorphisms may have an effect on cancer risk but also on sleep patterns and diurnal preference. Confirmation of some of these results in future studies may be useful before they can be used in screening or therapeutic techniques. In the MCC-Spain genotyping has been completed for breast, prostate and colorectal cases and controls. Circadian, sleep and genes regulating melatonin synthesis have been included and the genetic susceptibility for cancer as well as possible interactions with night shift work will be evaluated.

Only few studies have evaluated circadian disruption during pregnancy on birth outcomes while it has been hypothesized that

light-at-night (LAN) during pregnancy may have later effects in the offspring e.g. breast cancer. Effects of circadian and sleep disruption in childhood have also been poorly examined. Some of these hypothesis will be evalauted within mother-child cohorts in Greece (RHEA study) and Spain (INMA study), in children followed-up from pregnancy until 9-11 years. Sleep is being asessed prospectively in children at different ages with respect to outcomes such as growth, obesity, diabetes and cardiometabolic disease.

7. CONCLUSIONS

- Night shift work was associated with prostate cancer risk, particularly for tumors with worse prognosis.

- Night shift work was associated with breast cancer risk, particularly for women that had worked at night before their first full-term pregnancy.

- Chronotype was an effect modifier of the night shift work-cancer association. Evening types presented a higher breast and prostate cancer risk, compared to morning and neither types.

- Night shift workers experienced lower 6-sulfatoxymelatonin and higher progestagen and androgen 24 hour levels, compared to day workers.

- The peak time of 6-sulfatoxymelatonin and androgen production occurred later in night workers, compared to day workers.

- Lower melatonin, higher sex hormone levels and mistimed hormone production are mechanisms that may partly explain the increased risk for hormone-related cancers in night shift workers.

ANNEX

About the Author

Kyriaki Papantoniou received a degree in Medicine at the University of Patras, Greece in 2008 and a Master of Public Health at the Pompeu Fabra University and Autonoma University of Barcelona, Spain in 2010. She carried out her thesis in the Centre for Research in Environmental Epidemiology (CREAL) from 2010 to the present. As part of her PhD training, she did a 3-month stay (14th November 2011- 1st March 2012) at the Chronobiology group, University of Surrey, UK.

Published papers

Kyriaki Papantoniou and Manolis Kogevinas; Editorial: Shift work and breast cancer: do we need more evidence and what should this be? *Occup Environ Med 2013;70:12 825-826*

Papantoniou K, Pozo OJ, Espinosa A, Marcos J, Castaño-Vinyals G, Basagaña X, Calduch-Ribas F, Mirabent J, Martin J, Carenys G, Reyes-Martin C, Skene DJ, Kogevinas M. Circadian variation of melatonin, light exposure and diurnal preference in day and night shift workers of both sexes. *Cancer Epidemiol Biomark & Prev 2014; 23(7)*

Inés Gómez-Acebo, Trinidad Dierssen-Sotos, Kyriaki Papantoniou, María Teresa García-Unzueta, María Francisca Santos-Benito, Javier Llorca. Association between exposure to rotating night shift vs. day shift using levels of 6-sulfatoxymelatonin and cortisol and other sex hormones in women, *Accepted for publication in Chronobiology International.*

Submitted papers

Kyriaki Papantoniou, Oscar J. Pozo, Ana Espinosa, Josep Marcos, Gemma Castaño-Vinyals, Xavier Basagaña, Elena Juanola Pagès, Joan Mirabent, Jordi Martín, Patricia Such Faro Amparo, Gascó Aparici, Benita Middleton, Debra J. Skene and Manolis Kogevinas. Increased sex hormone production in male and female night shift workers. *Submitted to the J Pineal Res*.

Kyriaki Papantoniou, Gemma Castaño-Vinyals, Ana Espinosa, Nuria Aragonés, Beatriz Pérez-Gómez, Javier Burgos, Inés Gómez-Acebo, Javier Llorca, Rosana Peiró, Jose Juan Jimenez-Moleón, Francisco Arredondo, Adonina Tardón, Marina Pollan, Manolis Kogevinas. Night shift work, chronotype and prostate cancer risk in the MCC-Spain case-control study. *Submitted to the International Journal of Cancer.*

Papers in preparation

Kyriaki Papantoniou, Gemma Castaño-Vinyals, Ana Espinosa, Nuria Aragonés, Beatriz Pérez-Gómez, Jone M Altzibar, Inés Gómez-Acebo, Javier Llorca, Eva Ardanaz, Rosana Peiró, Juan Alguacil, Vicente Martin Sanchez, Adonina Tardón, Marina Pollan, Manolis Kogevinas. Breast cancer risk and night shift work in a case-control study in a Spanish population: the MCC-Spain study.

Kyriaki Papantoniou, Gemma Castaño-Vinyals, Victor Moreno, Ana Espinosa, Nuria Aragonés, Beatriz Pérez-Gómez, Jone M Altzibar, Inés Gómez-Acebo, Javier Llorca, Eva Ardanaz, Rosana Peiró, Juan Alguacil, Vicente Martin Sanchez, Adonina Tardón, Marina Pollan, Manolis Kogevinas. Night shift work and colorectal cancer risk in a population-based case-control study in Spain.

Published abstract: *Occup Environ Med. 2014 Jun;71 Suppl 1:A5-6.*
Link for abstract: http://www.ncbi.nlm.nih.gov/pubmed/25018382

Abstract

Objectives: Epidemiological cancer studies on shift work have focused on breast cancer while evidence on other tumours is limited. We evaluated colorectal cancer risk in relation to night and rotating shift work and genetic variation, in a population based case-control study in Spain.

Methods: 1066 male and 592 female incident colorectal cancer cases and 3388 randomly selected population controls of both sexes, enrolled in 11 regions of Spain, were included. Information was collected on socio-demographic, lifestyle, medical history and other variables by face-to-face interviews. Lifetime occupational history on daily time schedule of each job, day/night/rotating shifts, light at night exposure, and duration of different jobs, was used for exposure assessment. We used unconditional logistic regression adjusting for potential confounders.

Results: Among controls 10% of males and 4% females had ever worked full time in permanent night shifts (working between midnight and 6am) and 24% of males and 14% of females in rotating shifts for ≥1 year. Having ever performed rotating shift work was associated with an increased risk for colorectal cancer (adjusted Odds Ratio 1.33, 95% CI 1.15-1.55) compared to permanent day workers. ORs increased with cumulative years of rotating shift work and the OR for more than 30 years work 1.54 (1.22-1.94). Having ever worked in permanent night shift was not associated with colorectal cancer risk. Analysis on gene-environment interactions with genes in circadian, melatonin and sleep pathways are ongoing and will be presented.

Conclusions: In this large population based study we found an increase in colorectal cancer risk associated with rotating shift work.

Papantoniou Kyriaki , Guinó Elisabet , Castaño-Vinyals Gemma, Aragonés Nuria, Martin Sanchez Vincente, Gomez Acebo Inés, Jiménez Moleón José Juan, Peiro Rosana, Ardanaz Eva, Alguacil Juan, Altzibar Jone M., Tardón Antonina , María-Dolores Chirlaque , Kogevinas Manolis, Moreno Victor¸ Sleep and gastrointestinal cancer risk in the MCC-Spain case-control study.

Abstract

Objectives: Sleep duration is a modifiable risk factor associated with mortality and possibly cancer risk in humans. A few studies suggest that both long and short sleep may increase risk of colorectal cancer. In this large population based case control study we evaluated sleep duration but also sleep quality and timing with relation to gastrointestinal (colorectal and gastric) cancer risk in both sexes.

Methods: 1731 colorectal, 483 gastric cancer cases and 3940 randomly selected population controls, enrolled in 11 Spanish regions, were included. Information on habitual sleep duration, quality and timing, as well as shift work history, socio-demographic and lifestyle factors was collected by face-to-face interviews. We used unconditional logistic regression analysis adjusting for potential confounders.

Results: We found a U-shaped association between sleep duration and gastrointestinal cancer ($p<0.001$). Both long (≥9 hours) (OR 1.64; 95% CI 1.37-1.97) and short (≤5 hours) sleep duration (OR 1.12; 95% CI 0.94-1.35) increased gastrointestinal cancer risk, compared to 7 hours sleep per night. ORs for long sleep were 1.61 (95% CI 1.33-1.95) for colorectal and 1.77 (95% CI 1.27-2.47) for gastric cancer, respectively. Sleep onset after 2:00 h was associated with a higher risk for gastrointestinal cancer (OR 1.36; 95% CI 1.09-1.70).

Conclusions: In this large population based study we found an increased gastrointestinal cancer risk associated with both long and short sleep and late sleep onset.

Conference Presentations

21st International Symposium on Shiftwork and Working Time, *4-8/11/2013* Costa do Sauipe, Brazil; "Night shift-work, melatonin and sex hormones biomarkers" **(invited speaker)**

1st Young Researchers Conference on Environmental Epidemiology, *20-21/11/2014,* Barcelona, Spain*;* "Sleep and gastrointestinal cancer risk in the MCC-Spain case-control study" **(oral presentation)**

22nd Congress of the European Sleep Research Society, *16-20/09/2014,* Tallinn, Estonia; "Sleep and gastrointestinal cancer risk in the MCC-Spain case-control study" **(oral presentation)**

21st International Symposium on Shiftwork and Working Time, *4-8/11/2013* Costa do Sauipe, Brazil; "Shift work and colorectal cancer risk in a population-based case-control study in Spain" **(poster presentation)**

23rd Conference on Epidemiology in Occupational Health, EPICOH 2.0.13, Improving the Impact, *18-21/06/2013,* Utrecht, The Netherlands; "Melatonin and sex hormone biomarkers and light intensity exposure in female and male permanent night shift workers" **(oral presentation)**

Pineal Cell Biology, Gordon´s Research Conference, *29/01/2012-01/02/2012,* Galveston, Texas, USA; "Melatonin and sex hormone biomarkers and light measurements in female and male permanent night shift workers" **(oral presentation)**

23rd Conference of the International Society for Environmental Epidemiology (ISEE), *13-16/09/2011,* Barcelona, Spain; "Evaluation of breast cancer risk in relation to night shift work in a case-control study in a Spanish population" **(oral presentation)**

20th International Symposium on Shift work and Working Time, *28/06/2011-01/07/2011;* Stockholm, Sweden; "Evaluation of breast cancer risk in relation to night shift work in a case-control study in a Spanish population" **(oral presentation)**

Grants and Awards

2014: Travel grant, 22nd Congress of the European Sleep Research Society, *16-20/09/2014,* Tallinn, Estonia

2013: Travel grant, 21st International Symposium on Shiftwork and Working Time, *4-8/11/2013,* Costa do Sauipe, Brazil

2012: Travel grant, Pineal Cell Biology Gordon´s Research Conference, *29/01/2012-01/02/2012,* Galveston, Texas, USA

2011: Travel grant, 20th International Symposium on Shift work and Working Time, *28/06/2011-01/07/2011,* Stockholm, Sweden

2010: PhD fellowship: Instituto Carlos III de Madrid, FONDO DE INVESTIGACIÓN SANITARIA, FI09/00385, Instituto Carlos III (4 years)

2008: Lifelong Learning Programme-Leonardo da Vinci, EU (6 months)

Participation in Research Grant Proposals and Projects

Understanding the impact of circadian disruption on health, ageing and disease: a population and experimental approach (MIStiming); European Comission Horizon 2020 Call: H2020-PHC-2014-two-

stage, Topic: PHC-01-2014, PI KOGEVINAS MANOLIS/SEP-210176967; **SUBMITTED**

Estudio epidemiológico molecular sobre cambios hormonales asociados a la disrupción circadiana en trabajadores de turno de noche (HORMONIT) FIS/ISCIII - FONDO DE INVESTIGACIÓN SANITARIA. ISCIII; KOGEVINAS MANOLIS; **SUBMITTED**

Epigenetics of circadian rhythm disruption and breast cancer risk. FUNDACIÓ LA MARATÓ DE TV3¸ KOGEVINAS MANOLIS; 550910077-KOGEVINAS/MARATO TV3 -2013; **DENIED**

Estudio epidemiológico sobre el trabajo nocturno, disrupción del ritmo circadiano, susceptibilidad genética y riesgo de cáncer de mama y próstata; FIS/ISCIII - FONDO DE INVESTIGACIÓN SANITARIA. ISCIII; PI KOGEVINAS MANOLIS; 550307712-M. KOGEVINAS/FIS-11; **ONGOING 2012-01-01 to 2014-12-31.**

A molecular epidemiological study on shift work, changes in circadian rhythm and cancer risk, RECERCAIXA, 550307136-M. KOGEVINAS/RECERCAIXA-10; **DENIED**

Training seminars and teaching

Teaching seminars for the Environmental Sciences undergraduate programme of the Universitat Autonoma de Catalunya (4 hours per semester for 3 academic years)

Training seminar on “Night shift work and possible health risks” for superior technicians of the Occupational Health and Safety centre of the local government. (1 seminar)

Communicating science

Seminars for night shift workers of the participating companies, presenting study results and literature based general recommendations (2 seminars).

In collaboration with the Labour Department of the local government we redacted an information leaflet for night shift workers with literature review on health effects related to night shift work and recommendations for preventive actions.

Interview on the radio on “Night shift work and health risks”. Radio COPE, Spain, 07/07/2014; minute 17 to 28: www.cope.es/player/noche4080714&id=2014070805390001&activo=10

Reviewer

The author has been a reviewer for the following peer-reviewed scientific journals: the Occupational Environmental Medicine and Chronobiology International.

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