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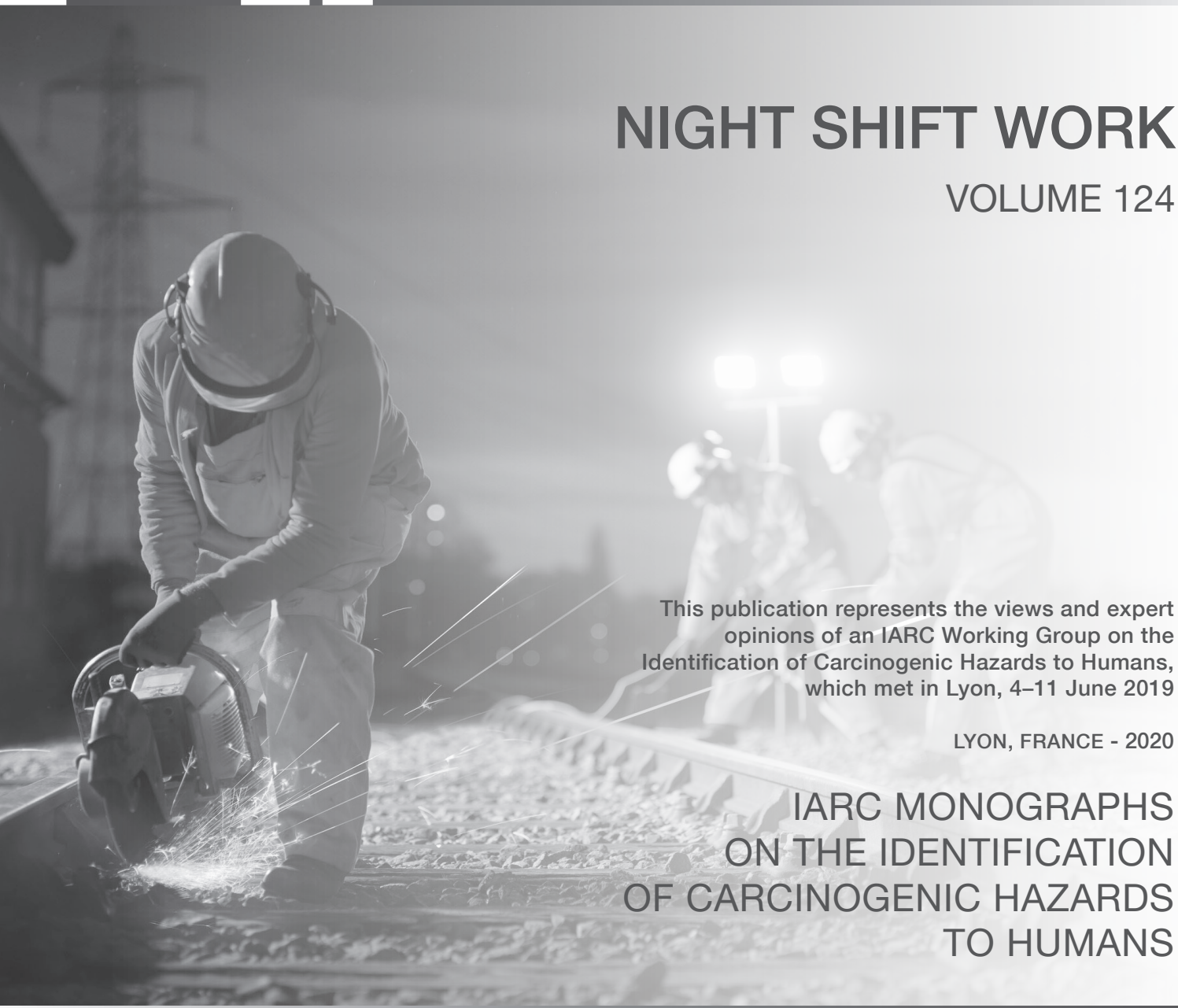

NIGHT SHIFT WORK

VOLUME 124

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4. MECHANISTIC EVIDENCE

4.1 Evidence relevant to key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016), including whether night shift work induces oxidative stress; is immunosuppressive; induces chronic inflammation; is genotoxic; induces epigenetic alterations; modulates receptor-mediated effects; alters cell proliferation, cell death or nutrient supply; and causes immortalization (i.e. induces changes in telomere length). For the evaluation of the other key characteristics of carcinogens, data were not available or considered insufficient.

4.1.1 Induces oxidative stress

(a) Humans

See Table 4.1.

In one study, levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in urine excreted by night shift workers during their day sleep were statistically significantly lower than those in urine excreted during their night sleep (Bhatti et al., 2016). Urinary 8-OHdG levels were not statistically significantly different between night shift workers and day shift workers. The study stipulated that participants were required to work at least 20 hours per week and 8 hours per shift, and that the night shift workers could not finish their shift earlier than 06:00. [The Working Group noted that in the reported comparison group, 8-OHdG levels were high.] In another study within the same population, the urinary 8-OHdG levels excreted by night shift workers were significantly lower during night work than during night sleep (Bhatti et al., 2017). In both studies, the results were adjusted for most of the relevant confounding covariates, such as age, sex, and alcohol consumption. Ishihara et al. (2008) reported that urinary 8-OHdG levels were significantly higher for female shift workers than for female part-time workers. [The Working Group noted that the comparison group was small and that the work schedules were ill defined. Also, the time-points for urine collection were not given.] Day–night (rotating) shift work in healthy men was correlated with increased urinary 8-OHdG levels ($P = 0.044$), but after adjustment for other lifestyle factors, this effect was borderline statistically significant (Kasai et al., 2001). An 11-fold inter-subject variation in 8-OHdG levels was observed, and detailed information on shift work schedules was lacking. [The Working Group noted that the findings on 8-OHdG were inconsistent across the four studies with regard to direction of effect.]

Manzella et al. (2015) reported lower expression of the 8-oxoguanine DNA glycosylase-1 (*OGG1*) gene, which is responsible for excising oxidized guanine, in night shift workers than in day shift workers. However, the shift work conditions of the comparison group (factory workers) were unclear. In a cross-sectional study of 49 full-time doctors, participants who worked on call,

Table 4.1 Oxidative stress in night shift workers

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Reference
8-OHdG	Urine	USA Health-care industry workers Cross-sectional study	440 (217 DS, 223 NS)	8-OHdG in NS group during day sleep period < night sleep period ($P = 0.03$)	Age, sex, alcohol consumption in the 24 h preceding the urine collection period	Bhatti et al. (2016)
8-OHdG	Urine	USA Health-care industry workers Short-term follow-up study	50 (NS)	8-OHdG in night work period < night sleep period ($P < 0.001$)	Age, sex	Bhatti et al. (2017)
8-OHdG	Urine	Japan Nurses, office workers, etc. Cross-sectional study	77 (65 F NS, 12 F part-time DS)	NS > part-time DS ($P < 0.004$)	None	Ishihara et al. (2008)
8-OHdG	Urine	Japan Steel-manufacturing company Cross-sectional study	318 (169 day-night shift, 149 DS)	Day-night shift > DS ($P = 0.055$)	Age, BMI, smoking, meat consumption (number of times per week), exercise	Kasai et al. (2001)
OGG1 gene expression	Lymphocytes	Italy Nurses Cross-sectional study	116 (60 NS, 56 DS)	NS < DS ($P < 0.05$)	None	Manzella et al. (2015)
DNA strand breaks (FPG+ comet assay); DNA repair gene expression (*OGG1*, *ERCC1*, *XRCC1*)	Blood	Hong Kong Special Administrative Region Medical doctors Cross-sectional study	49 (24 on-call on-site working overnight, 25 with no overnight work)	DNA breaks (+ and – FPG) in NS > DS ($P < 0.0001$) DNA repair in NS < DS ($P < 0.01$)	None Multiple eligibility criteria	Cheung et al. (2019)
SOD, CAT, MDA	Blood	Spain Intensive-care nurses Cross-sectional	32 nurses (7 M, 25 F) and 35 age-matched workers (12 M, 23 F). SW: MS, ES, NS	SOD and MDA for ES and NS > DS ($P < 0.01$)	Multiple eligibility criteria	Casado et al. (2008)

Table 4.1 (continued)

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Reference
SOD, CAT, MDA	Blood	Spain Palliative-care workers Cross-sectional	52 nurses (23 M, 29 F) and 50 age-matched workers (24 M, 26 F). SW: MS, ES, NS	SOD and MDA for ES and NS > DS ($P < 0.01$)	Multiple eligibility criteria	Casado et al. (2011)
d-ROM, BAP	Blood	Japan Local government prison officers Cross-sectional	55 F SW, 63 F DS	BAP for SW > DS ($P < 0.0001$) d-ROM/BAP for SW < DS ($P = 0.001$)	Age, BMI, communication time, duration of sleep, alcohol consumption, smoking, VDT time	Ebata et al. (2017)
TOS, OSI, TAS	Blood	Turkey Medical residents, nurses, non-health-care staff Cross-sectional	71 medical residents (55 M, 16 F) on 24-h shift, and 45 nurses (F) and 30 (15 M, 15 F) non-health-care staff working 8-h shift	TOS and OSI were increased and TAS decreased ($P < 0.001$) after 24-h continuous shifts compared with 8-h shift	Within-person analysis	Buyukhatipoglu et al. (2010)
8-Isoprostane	Urine	Japan Breast-cancer screening survey Cross-sectional	F NS ($n = 10$) and non-shift workers ($n = 532$)	NS > non-shift ($P = 0.03$)	Age, BMI, smoking status, physical activity, use of dietary supplements, history of hypertension and diabetes	Nagata et al. (2017)
TAC	Blood	Islamic Republic of Iran Industrial catering staff Cross-sectional	44 RS (day–night–off–off)	DS < NS ($P < 0.001$)	Age, BMI Multiple eligibility criteria	Sharifian et al. (2005)
GSH-Px	Blood	Poland Nurses Cross-sectional	349 rotating NS, 359 DS only	Rotating NS > DS ($P = 0.009$)	Age, oral contraceptive hormone use, smoking, alcohol consumption during last 24 h	Gromadzińska et al. (2013)
TOS, TAS, OSI	Blood collected before and after shift	Turkey Nurses Cross-sectional	60 NS, 60 DS	Significant increase at the end of the shift (in both NS and DS)	None Multiple eligibility criteria	Ulas et al. (2012)
TOS, TAS, OSI	Blood collected before and after shift	Turkey Nurses Cross-sectional	70 NS, 70 DS	Significant increase at the end of the shift (in both NS and DS)	None Multiple eligibility criteria	Ulas et al. (2013)

Table 4.1 (continued)

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Reference
CAT, TTG, TAC	Saliva	Islamic Republic of Iran Control room operators (petrochemical complex) Cross-sectional	30 RS	– (for various light conditions)	Multiple eligibility criteria	Kazemi et al. (2018)
TAC, MDA	Blood	Islamic Republic of Iran Refinery workers Cross-sectional	189 RS	– (for SW)	SW history Multiple eligibility criteria	Khajehnasiri et al. (2014)
TAC, TOS, OSI	Blood	Turkey Health-care workers Cross-sectional	45 rotating NS, 45 DS	OSI, NS > DS (P = 0.051) – (TOS, TAC)	None Multiple eligibility criteria	Özdemir et al. (2013)

–, not significant; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; BAP, biological antioxidant potential; BMI, body mass index; CAT, catalase; d-ROM, reactive oxygen metabolites-derived compound; DS, day shift; ERCC1, excision repair 1, endonuclease non-catalytic subunit; ES, evening shift; F, female; FPG, formamidopyrimidine-DNA glycosylase; GSH-Px, glutathione peroxidase; h, hour; M, male; MDA, malondialdehyde; MS, morning shift; NS, night shift; OGG1, 8-oxoguanine DNA glycosylase 1; OSI, oxidative stress index (TOS/TAC); RS, rotating shift; SOD, superoxide dismutase; SW, shift work; TAC, total antioxidant capacity; TAS, total antioxidant status; TOS, total oxidant status; TTG, total thiol molecules; VDT, video display terminal; XRCC1, X-ray repair cross complementing 1.

overnight, and on site with acute sleep deprivation, defined as sleeping less than two sleep cycles (3 hours) during their shift, had lower baseline expression of DNA repair genes and more oxidative damage to DNA than participants who did not work overnight (Cheung et al., 2019).

More than 10 studies have evaluated oxidative stress biomarkers other than 8-OHdG in blood, serum, saliva, or urine (see Table 4.1). These biomarkers included superoxide dismutase (SOD), catalase (CAT), oxidized low-density lipoprotein, malondialdehyde (MDA), neutrophil gelatinase lipocalin-2, glutathione peroxidase (GSH-Px), serum prolidase, reactive oxygen metabolite-derived compounds (d-ROMs), biological antioxidant potential (BAP), total thiol molecules, glycosaminoglycans, and 8-isoprostane. The studies also measured total oxidative status (TOS), total antioxidant status (TAS), total antioxidant capacity (TAC), and total oxidative stress index (OSI). [The Working Group noted that few studies measured more than one of these markers, and replicated results were not always available for the same end-point across studies.]

Seven studies reported positive associations. In two studies, significantly increased levels of the oxidative stress biomarkers SOD and MDA were found in shift workers working night and evening shifts compared with those working day shifts (Casado et al., 2008, 2011). There was no effect of sex, but the oxidative stress levels increased with age. In another study, BAP levels were significantly increased, and the ratio of d-ROMs to BAP was reduced, in female night shift workers compared with female daytime workers (Ebata et al., 2017). The results were corrected for multiple covariates, including age, body mass index (BMI), sleep duration, smoking, and alcohol consumption. These studies had a small sample size and no detailed exposure information, and time of blood collection was not reported. In another small study, 24-hour work shifts resulted in increased TOS levels and decreased TAS in blood compared with 8-hour work shifts (Buyukhatipoglu et al., 2010). Furthermore, women currently working night shifts had significantly higher levels of the oxidative stress biomarker 8-isoprostane in urine compared with women not working night shifts (Nagata et al., 2017). [The Working Group noted that the number of current night shift workers included was small (n = 10).] In a small group of shift workers in industrial catering, rotating from day shift work to night shift work was associated with a significant reduction in total plasma antioxidant capacity, in a study that assessed the impact of age and BMI (Sharifian et al., 2005). In a cross-sectional study of rotating shift workers, the GSH-Px activity measured in erythrocytes of premenopausal nurses was significantly higher in those working rotating night shifts than in those working only day shifts (Gromadzińska et al., 2013). This increase in the levels of GSH-Px activity was associated with working more night shifts per month. High GSH-Px activity may be indicative of a higher oxidative stress level. Data were corrected for age, use of hormonal oral contraceptives, smoking, and alcohol consumption during the previous 24 hours.

Five studies reported no significant associations between night shift work and oxidative stress markers. Ulas et al. (2012, 2013) compared oxidative stress levels measured at two time-points (08:00 and 16:00) and found that the levels were significantly increased in all nurses at the end of both the day shift and the night shift. However, no comparisons were made between day shift workers and night shift workers. Kazemi et al. (2018) found no associations between oxidative stress and various light intensities, but the intensity and duration of night shift work were not described. Khajehnasiri et al. (2014) reported no significant association between shift work experience and levels of TAC and MDA in rotating shift workers in an oil refinery. Özdemir et al. (2013) reported no differences in the oxidative stress status (TOS and TAC) between day shift workers and night shift workers, but an increased

OSI was found in night shift workers, which was borderline statistically significant.

[The Working Group noted that the majority of the studies were cross-sectional in design and some had insufficient details on shift work schedules, some were lacking specific information on the intensity and duration of night shift work, some had small sample sizes, and in some the biosamples were collected at only one time-point.]

(b) Experimental systems

Data from studies investigating whether oxidative stress is induced after alterations in the light–dark schedule are compiled in Table 4.2. A short-term advance in the light–dark schedule in male stroke-prone spontaneously hypertensive rats (SHRSP) and in male Wistar-Kyoto (WKY) rats was associated with increased levels of thiobarbituric acid-reactive substances in the rostral ventrolateral medulla (Kishi & Sunagawa, 2011). Levels of oxidative stress markers (e.g. lipid peroxidation) were also elevated in male Wistar rats exposed to continuous (23.5 hours) dark conditions for 30 days (Kuchukashvili et al., 2012). Increased expression of antioxidant systems (Cu,Zn-SOD; MnSOD; and extracellular SOD) was also reported in male Wistar rats housed under continuous light for 6 weeks (Temneanu et al., 2012). In mice that underwent shifts in the light–dark schedule for 10 days, glutathione levels decreased and nicotinamide adenine dinucleotide levels increased in the brain (LeVault et al., 2016). Nocturnal adult female tammar wallabies housed for 10 weeks under either amber light or white light at night had no change in plasma lipid peroxidation levels (for both light types) and decreased plasma antioxidant capacity (for both light types) when compared with animals housed under continuous dark conditions at night (Dimovski & Robert, 2018; see also Section 4.2.1(b)).

Alterations in the light–dark schedule in young diurnal Sudanian grass rats (*Arvicanthis ansorgei*) for 3 months did not alter either plasma antioxidant capacity or hepatic 8-OHdG levels (Grosbellet et al., 2015; see also Section 4.1.8(b)).

4.1.2 Is immunosuppressive

(a) Humans

Several studies have evaluated the occurrence of infectious disease in association with shift work (Mohren et al., 2002; Boden et al., 2014; Vijayalaxmi et al., 2014; Loef et al., 2019). While all were based on self-report of infection status, each of the studies has demonstrated an increased occurrence of infection in association with shift work. For example, in a study of 501 rotating and/or night shift workers and 88 non-shift workers, the incidence of self-reported influenza-like illness and acute respiratory infection in shift workers was 1.2-fold (95% confidence interval (CI), 1.01–1.43) that in non-shift workers (Loef et al., 2019). [The Working Group noted that suppression of immune function is a potential mechanism that may underlie these observed associations.]

Multiple studies evaluating markers of immune function in association with shift work were identified and are summarized in Table 4.3 and Table 4.4. Most of the studies were cross-sectional in nature and compared various measures of immune function between day shift workers and either permanent night shift workers or rotating shift workers (see Table 4.3). The largest study involved participants in the National Health and Nutrition Examination Survey (NHANES) (Buss et al., 2018). No statistically significant differences in lymphocyte count were observed when comparing day shift workers with night shift or rotating shift workers. Details with regard to frequency, intensity, and duration of shift work were not captured. In addition, only blood samples collected at a single time-point were compared and the timing of blood collection was not specified. Measures of immunity, such as lymphocyte count, vary diurnally (Fan et al., 1977; Cove-Smith et al., 1978; Bertouch

Table 4.2 Oxidative stress after alterations in the light–dark schedule in experimental systems

Experimental system	Exposure	Relevant findings	Reference
Adult SHRSP and WKY rat (M)	Control: LD14:10 Treatment: 12-h advance in the light–dark schedule for 5 d	WKY: ↑ TBARS in the RVLM (day 2) SHRSP: ↑ TBARS in the RVLM (days 2 and 5)	Kishi & Sunagawa (2011)
Wistar rat (M)	Control: LD10:14 Treatment: continuous (23.5 h) darkness for 30 d	In brain, ↑ lipid peroxidation and oxidative stress (NO, Ca^{2+}, MDA, diene conjugates) and ↓ mitochondrial SOD and catalase activity	Kuchukashvili et al. (2012)
Wistar rat (M)	Control: LD12:12 Treatment: continuous light for 6 wk	↑ Lung Cu,Zn-SOD, Mn-SOD, and extracellular SOD expression	Temneanu et al. (2012)
APPSwDI *NOS2*$^{-/-}$ mouse (M, F) Non-transgenic mouse on C57BL/6 109 background (M, F)	Control: LD12:12 Treatment: 8-h advance in the light–dark schedule every 3 d for 10 d	↓ Brain GSH and ↑ NADH levels in both genotypes	LeVault et al. (2016)
Adult tammar wallaby (*Macropus eugenii*) (F)	Control: no light during the period of darkness (0.37×10^{-3} W/m^2) Treatment: amber LED (605 nm, 2.00 W/m^2), or white LED (448 nm, 2.87 W/m^2) during the period of darkness for up to 10 wk	White LED during the period of darkness: ↓ plasma melatonin concentration White or amber light during the period of darkness: no change in plasma lipid peroxidation levels; ↓ plasma Trolox equivalent antioxidant capacity at 10 wk	Dimovski & Robert (2018) (see also Section 4.2.1(b))
Sudanian grass rat (M) (diurnal)	Control: LD12:12 for 3 mo Treatment: advance in the light–dark schedule by 10 h every week for first 4 d of the week for 3 mo	No difference in oxidative damage to DNA (8-OHdG levels, ELISA) in liver or plasma antioxidant capacity (OXY) Shorter telomeres (qPCR) in the liver, not only in old rat liver cells (–18%) but also at an intermediate level in shifted young rats (–9%)	Grosbellet et al. (2015) (see also Section 4.1.8(b))

↑, increase; ↓, decrease; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; d, day; ELISA, enzyme-linked immunosorbent assay; F, female; GSH, glutathione; h, hour; LD, light–dark schedule, light(h):darkness(h); LED, light-emitting diode; M, male; MDA, malondialdehyde; mo, month; NADH, nicotinamide adenine dinucleotide; NO, nitric oxide; NOS2$^{-/-}$, nitric oxide synthase 2$^{-/-}$; qPCR, quantitative polymerase chain reaction; RVLM, rostral ventrolateral medulla; SOD, superoxide dismutase; SHRSP, stroke-prone spontaneously hypertensive; TBARS, thiobarbituric acid-reactive substances; wk, week; WKY, Wistar-Kyoto.

Table 4.3 End-points relevant to immune function in cross-sectional studies in shift workers

End-point	Location, setting	Exposure level, and number of exposed and controls	Response (significance)	Covariates controlled	Reference
Lymphocyte count	USA NHANES	6667 DS, 970 NS/ES, 809 RS	–	Age, race, education, marital status, diabetes, smoking status, cold illness, waist circumference, HDL, total cholesterol, systolic blood pressure, BMI	Buss et al. (2018)
Lymphocyte count	Japan Male and female physicians at critical-care emergency centres and hospitals	27 RS (8–12 h including NS), 39 traditional shift (includes NS), 8 DS	–	None	Okamoto et al. (2008)
Lymphocyte count	Germany Male and female industrial workers	137 DS, 225 shift workers (91% in 3-shift RS with NS; 9% in other including permanent NS)	–	None	van Mark et al. (2010)
Lymphocyte count	Italy Hospital nurses	28 DS, 68 RS (1 DS 06:00–14:00; 1 AS 14:00–22:00; 1 NS 22:00–06:00), ≥ 2 yr in current schedule	–	Job seniority, and presence of offspring	Copertaro et al. (2011)
Lymphocyte proliferative response	Italy Hospital nurses	28 DS, 68 RS (1 DS 06:00–14:00; 1 AS 14:00–22:00; 1 NS 22:00–06:00), ≥ 2 yr in current schedule	–	Job seniority, and presence of offspring	Copertaro et al. (2011)
Lymphocyte proliferative response	Italy Male pressmen in newspaper industry	12 AS, 12 RS (weekly rotation: DS 06:00–13:00; NS 23:00–06:00; AS, 13:00–20:00)	RS < AS ($P < 0.025$)	Multiple eligibility criteria	Curti et al. (1982)
T-cell proliferative response	Japan Wholesale market	20 NS, 19 DS	NS < DS ($P < 0.05$)	None	Nakano et al. (1982)
T-cell proliferative response	Japan Iron-manufacturing company	20 RS (DS 07:00–15:00; AS 15:00–22:00; NS 22:00–07:00), 20 DS	RS < DS at 17:00 after DS ($P < 0.05$) and at 19:00 at start of NS ($P < 0.05$)	None	Nakano et al. (1982)

Table 4.3 (continued)

End-point	Location, setting	Exposure level, and number of exposed and controls	Response (significance)	Covariates controlled	Reference
NK cell activity	Japan Male and female physicians at critical-care emergency centres and hospitals	27 RS (8–12 h, including NS), 39 traditional shift (includes NS), 8 DS	RS< DS ($P < 0.01$)	None	Okamoto et al. (2008)
NK cytotoxicity	Italy Hospital nurses	28 DS, 68 RS (1 DS 06:00–14:00; 1 AS 14:00–22:00; 1 NS 22:00–06:00), ≥ 2 yr in current schedule	–	Job seniority, and presence of offspring	Copertaro et al. (2011)

–, not significant; AS, afternoon shift; BMI, body mass index; DS, day shift; ES, evening shift; h, hour; HDL, high-density lipoprotein; NHANES, National Health and Nutrition Examination Survey; NK, natural killer; NS, night shift; RS, rotating shift; yr, year.

Table 4.4 End-points relevant to immune function in non-cross-sectional studies in shift workers

End-point[a]	Location, setting	Exposure level, and number of exposed and controls	Response (significance)	Covariates controlled	Reference
Lymphocyte count	Islamic Republic of Iran NR	25 randomized to 3 DS, 1 rest day, and 3 NS; 25 randomized to 3 NS, 1 rest day, and 3 DS	NS > DS ($P = 0.008$)	Multiple eligibility criteria; within-person analysis	Khosro et al. (2011)
Lymphocyte count	Japan Female nurses at hospital	57 RS (DS 08:30–17:15; NS 00:30–09:15; ES 16:30–01:15), mean 19 yr duration	NS > DS for CD3 and CD4 ($P < 0.01$); NS < DS CD16/CD56 ($P < 0.01$)	Within-person analysis	Nagai et al. (2011)
NK cell activity	Japan Female nurses at hospital	57 RS (DS 08:30–17:15; NS 00:30–09:15; ES 16:30–01:15), mean 19 yr duration	NS < DS for NK cell activity ($P < 0.01$)	Within-person analysis	Nagai et al. (2011)
Cytokine secretion/ T-lymphocyte	Canada Healthy men and women in laboratory study	8 participants transitioned from DS to NS	Phase advance in IFNγ and IL2 ($P < 0.001$)	Multiple eligibility criteria; within-person analysis	Cuesta et al. (2016)
PBMC transcriptome	Canada Healthy men and women in laboratory study	8 participants transitioned from DS to NS	Phase shift and/or reduced amplitude of NK immune response transcripts	Multiple eligibility criteria; within-person analysis	Kervezee et al. (2018)
Proteome	USA Men in laboratory study	6 participants transitioned from DS to NS	NS < DS for antigen presentation and IFN signalling proteins ($P < 0.05$)	Multiple eligibility criteria; within-person analysis	Depner et al. (2018)

DS, day shift; ES, evening shift; IL, interleukin; IFN, interferon; NK, natural killer; NR, not reported; NS, night shift; PBMC, peripheral blood mononuclear cell; RS, rotating shift.

[a] End-points measured in blood.

et al., 1983) [the Working Group noted that single time-points of comparison could miss effects that occur at other times of the day, and systematic differences in blood collection timing could lead to spurious associations or even mask significant associations]. No significant associations with lymphocyte count were observed in any of the other cross-sectional studies that evaluated this marker (Okamoto et al., 2008; van Mark et al., 2010; Copertaro et al., 2011). A few cross-sectional studies evaluated other markers, including lymphocyte or T-cell proliferative response and natural killer (NK) activity and/or cytotoxicity, and findings were mixed (Curti et al., 1982; Nakano et al., 1982; Okamoto et al., 2008; Copertaro et al., 2011).

Although most of the cross-sectional studies included well-defined timings of blood sample collection, they had various other limitations, including small sample sizes, minimal to no adjustment for covariates, different blood collection times for day shift workers versus and night or rotating shift workers, a lack of data on duration of work for the various shift schedules, and unspecified temporal proximity or no temporal proximity to night shift work (Curti et al., 1982; Nakano et al., 1982; Okamoto et al., 2008; van Mark et al., 2010; Copertaro et al., 2011). [The Working Group noted that the latter is unlikely to be an issue for detecting longer-term (i.e. chronic) effects of night shift work.] For example, in their study of 68 rotating shift workers and 28 day shift workers who had worked their respective shifts for at least 2 years, Copertaro et al. (2011) observed no significant differences in lymphocyte count, lymphocyte proliferative response, or NK cell cytotoxicity between the two groups. However, blood samples for the rotating shift workers were collected on a day off with no night shift work on the preceding day. [The Working Group noted that acute effects of night shift work may have been missed because of the recovery of immune function over multiple days without night shift work.]

Of the remaining studies, summarized in Table 4.4, one was a randomized crossover trial (Khosro et al., 2011), another was a short-term longitudinal study (Nagai et al., 2011), and two were small but highly controlled studies conducted in sleep laboratories (Cuesta et al., 2016; Depner et al., 2018). All evaluated very short periods of exposure to shift work, meaning that the studies were more likely to detect acute as opposed to chronic effects. In the crossover trial, lymphocyte counts were evaluated in 50 participants who were randomized to either start with 3 day shifts and then transition to 3 night shifts, or start with 3 night shifts and then transition to 3 day shifts (Khosro et al., 2011). Blood samples were collected in the morning during both day shift and night shift periods. Night shift work was associated with an elevated lymphocyte count. In the short-term longitudinal study, immune system measures were compared in a group of rotating shift workers using blood samples collected on consecutive days (in the morning on a day shift and in the morning after completing a night shift) (Nagai et al., 2011); no other time-points were captured. Statistically significantly increased counts of CD3 and CD4 and decreased counts of CD16/56 lymphocytes were observed after night shift work. In one of the laboratory-based studies, eight healthy participants were transitioned from a day shift to a night shift schedule (maintained for only 4 days), with multiple blood samples collected during the simulated day shift and night shift periods (Cuesta et al., 2016). Transitioning to a night shift schedule resulted in statistically significant phase advances in the per-cell secretion of interferon-gamma (IFNγ) and interleukin 2 (IL2) in stimulated T lymphocytes. Using samples from the same study, the impact of a night shift schedule on the blood transcriptome was investigated (Kervezee et al., 2018). A phase shift and reduced amplitude of transcripts related to NK cell-mediated immune response was observed during the night shift. In a separate laboratory-based study

of proteomics in six healthy participants who were transitioned from a day shift to a night shift schedule (maintained for only 2 days), a decrease in proteins associated with antigen presentation and processing and interferon signalling was observed (Depner et al., 2018).

(b) Experimental systems

See Table 4.5.

Multiple studies have demonstrated suppression of the immune response in rodents exposed to alterations in the light–dark schedule. Logan et al. (2012) investigated the effects of shifts in the light–dark schedule on NK cell function in male Fischer 344 rats. NK cytotoxicity was reduced in the group exposed to shifts in the light–dark schedule, and the suppressed circadian expression of NK cell cytolytic activity was associated with increased growth of tumours after intravenous injection of mammary adenocarcinoma cells. Shifts in the light–dark schedule affected the circadian expression of the cytolytic factors perforin and granzyme B, and IFNγ, all critical factors in the cytotoxicity of NK cells. In a study of immune function in male Sprague-Dawley rats, Li & Xu (1997) showed that alteration of the light–dark schedule modulated the circadian pattern of the delayed-type hypersensitivity (DTH) response to sheep red blood cells (SRBCs) and suppressed the response at multiple timepoints. Phagocytosis was also suppressed in rats exposed to shifts in the light–dark schedule (Li & Xu, 1997). Valdés-Tovar et al. (2015) demonstrated suppression of the primary antibody response to SRBC in male Wistar rats exposed to continuous light for 15 days. A 38% decrease in the number of IL2-secreting splenocytes was also observed, and corresponded to decreased secretion of IL2 when splenocytes taken from continuous light exposed rats were stimulated with SRBC in vitro. Secretion of immunoactive Met-enkephalin-containing peptides, which modulate macrophage activation and cytokine production, was also reduced in these cells. In contrast, in diurnal Nile grass rats, dim light during the period of darkness enhanced innate, cell-mediated, and humoral immunity (Fonken et al., 2012). DTH responses after sensitization with 2,4-dinitro-1-fluorobenzene (DNFB), antigen-specific immunoglobulin-G in the plasma 10 and 15 days after immunization with keyhole limpet haemocyanin in male rats, and bactericidal activity against *Escherichia coli* were all elevated in rats exposed to dim light during the period of darkness compared with controls (Fonken et al., 2012). Deprés-Brummer et al. (1997) reported that in male Sprague-Dawley rats, leukocytes and certain lymphocyte subpopulations showed circadian patterns, which could be modulated by prolonged exposure to continuous light or continuous darkness. Exposure to continuous darkness increased the absolute number of circulating T and B lymphocytes in male Wistar rats (Mikolajczak et al., 2000).

Viral clearance after intranasal infection with a murine gammaherpesvirus and response to a secondary inflammatory challenge with lipopolysaccharide (LPS) was investigated in male and female BALB/cByJ and *IFNγ*-deficient BALB/cByJ mice exposed to shifts in the light–dark schedule (Trammell & Toth, 2016). Although there was no difference in viral clearance in multiple tissues after the initial infection, viral load was significantly increased in the lungs of in BALB/cByJ mice exposed to shifts in the light–dark schedule, 7 days after LPS treatment. *IFNγ* knock-out mice exposed to shifts in the light–dark schedule had lower levels of pulmonary cytokines than control mice (Trammell & Toth, 2016). Alteration in the daily light–dark schedule modulated plasma cytokine levels in response to LPS injection (Phillips et al., 2015). Basal levels of plasma cytokines were similar; however, 3 hours after LPS injection, alteration of the light-dark schedule resulted in a significant increase in plasma IL17, a suppression of plasma IL6, IL12, monocyte chemoattractant protein-1, and keratinocyte chemoattractant, as well as a significant

Table 4.5 Immune suppression in response to alterations in the light–dark schedule in experimental systems

Experimental system (sex)	Exposure	Relevant finding(s)	Reference
Fischer 344 rat (M)	Control: LD12:12 Treatment: repeated 6-h advance in the light–dark schedule every 2 d for 10 d, followed by continuous darkness for 5–7 d; injection with MADB106 mammary adenocarcinoma cells at CT 19 during the period of continuous darkness	↓ NK cell cytotoxicity Altered expression of cytolytic factors (perforin, IFNγ, granzyme B) ↑ Lung tumour growth (MADB106 mammary adenocarcinoma)	Logan et al. (2012) (see also Section 4.2.2)
Sprague-Dawley rat (M)	Control: LD12:12 Treatment: LD14:10 for 3 d then LD10:14 for 3 d, for a total of 28 d	↓ DTH response ↓ Phagocytosis	Li & Xu 1997
Wistar rat (M)	Control: LD12:12 Treatment: continuous light (50 lux) for 15 d	↓ Humoral immune response after immunization with sheep red blood cells ↓ Number of IL2 secreting splenocytes ↓ IL2 secretion after antigen stimulation ex vivo	Valdés-Tovar et al. (2015)
Nile grass rat (*Arvicanthis niloticus*) (M) (diurnal)	Control: LD14:10 Treatment: LD14:10, with dim light (5 lux) during the period of darkness for 3 wk	↑ Anti KLH IgG in plasma ↑ DTH after DNFB ↑ Plasma bactericidal activity against *Escherichia coli* in vitro	Fonken et al. (2012)
Sprague-Dawley rat (M)	Control: LD12:12 Treatment: continuous light for 8 wk, then continuous darkness for 2 wk Treatment: continuous light or continuous darkness for 17 wk, then return to LD12:12 for 16 wk	Mean counts of circulating leukocytes were similar in all groups at 8 wk Leukocyte circadian rhythm suppressed after continuous light for 11 or 16 wk, with persistent loss of synchronization after return to LD12:12 Leukocyte circadian rhythm suppressed after 16 wk of continuous darkness, with reversion to normal synchronization after return to LD12:12 ↓ NK cells after continuous light for 11 or 16 wk, which persisted after return to LD12:12	Deprés-Brummer et al. (1997)
Wistar rat (M)	Control: LD12:12 Treatment: continuous darkness for 12 wk	↑ Number of circulating T lymphocytes (both $CD4^+$ and $CD8^+$) ↑ Number of circulating B lymphocytes	Mikolajczak et al. (2000)
BALB/cByJ mouse (M, F) *IFNγ*-deficient BALB/cByJ mouse	Control: LD12:12 Treatments: 8-h extension of period of darkness for 5 d for study duration, then 2-d rest period with LD12:12 for 10 wk Intranasal infection with murine gammaherpes virus	No significant difference in viral clearance after initial infection ↑ Viral load in lungs of BALB/cByJ mice after LPS ↓ Pulmonary cytokines in *IFNγ*-deficient mice	Trammell & Toth (2016)
C57BL/6 mouse (M)	Control: LD12:12 Treatment: LD10:10 for 4 wk	Altered plasma cytokine in response to LPS (400 µg/kg bw) challenge Altered cytokine mRNA expression in hypothalamus	Phillips et al. (2015)

Table 4.5 (continued)

Experimental system (sex)	Exposure	Relevant finding(s)	Reference
C57BL/6 mouse (M)	Control: LD12:12 Treatments: 6-h advance in the light–dark schedule for 4 wk or continuous darkness for 2 d 3.5-mm punch biopsy wound	↑ Peripheral blood levels of IL6 after ex vivo culture with LPS	Adams et al. (2013)
C57BL/6 mouse (M)	Control: LD12:12 Treatment: advance in the period of light by 8 h every 48 h for 2 wk	↑ Plasma IL6	Wu et al. (2010)
Kunming mouse (M)	Control: LD12:12 Treatment: LD14:10 for 3 d, then LD10:14 for 3 d, and injection of Ehrlich carcinoma cells at onset of altered light–dark schedule	↓ Total leukocytes ↓ Lymphocytes ↓ Survival after tumour transplantation	Li & Xu 1997
C57BL/6 mouse (F)	Control: LD14:10 Treatment: dim light (5 lux) during period of darkness for 3 wk	Exposure to dim light before wounding delayed healing and time to wound closure	Walker et al. (2019)
Siberian hamster (*Phodopus sungorus*) (M)	Control: LD16:8 Treatment: dim light (5 lux) during period of darkness for 4 wk	↓ Cell-mediated immune responses to DNFB ↓ Bactericidal activity in plasma after LPS (400 µg/kg bw) challenge	Bedrosian et al. (2011)
Siberian hamster (*Phodopus sungorus*) (M)	Control: LD16:8 Treatment: LD8:16 for approximately 20 wk	Altered febrile response after LPS (400 µg/kg bw) challenge No significant difference in cell-mediated immunity	Ikeno et al. (2014)
Siberian hamster (*Phodopus sungorus*) (F)	Control: LD16:8 Treatment: single 2-h light pulse, then 3-h alteration in the light–dark schedule by extending the period of light	↓ Febrile response to LPS (625 µg/kg bw) after a 30-d recovery period	Prendergast et al. (2015)
Siberian hamster (*Phodopus sungorus*) (F)	Control: LD16:8 Sham control: LD16:8 then 3-h alteration in the light–dark schedule by extending the period of light (no single 2-h light pulse) Treatment: single 2-h light pulse, then 3-h alteration in the light–dark schedule by extending the period of light 3.5-mm punch biopsy wound	Delayed wound healing	Cable et al. (2017)
Siberian hamster (*Phodopus sungorus*) (M)	Treatments: long day, LD16:8; short day, LD8:16; long day, LD16:8 with dim light (5 lux) during period of darkness; short day, LD8:16 with dim light (5 lux) during period of darkness for 8 wk Restraint stress: 2 wk at the end of the alteration in the light–dark schedule	Dim light during the period of darkness: ↓ cell-mediated immune responses to DNFB ↓ Cell-mediated immunity, exacerbated by restraint stress	Aubrecht et al. (2014)

↑, increase; ↓, decrease; bw, body weight; CT, circadian time; d, day; DNFB, 2,4-dinitro-1-fluorobenzene; DTH, delayed-type hypersensitivity; F, female; h, hour; IFN, interferon; IgG, immunoglobulin-G; IL, interleukin; KLH, keyhole limpet haemocyanin; LD, light-dark schedule, light(h):darkness(h); LPS, lipopolysaccharide; M, male; mRNA, messenger RNA; NK, natural killer; wk, week.

elevation of mRNA expression of *IL6* and tumour necrosis factor alpha (*TNFα*) in the hypothalamus. Adams et al. (2013) evaluated the response to an inflammatory challenge in male C57BL/6 mice exposed to continuous darkness for 2 days. LPS-induced IL6 secretion was elevated at most time-points examined, although it followed a release pattern similar to that of controls. In the same study, serum IL6 was elevated in mice exposed to an altered light–dark schedule for 4 weeks. Advancing the onset of the period of light changed the circadian profiles of peripheral lymphocytes and T-helper cells in the spleen, and increased plasma IL6 levels in male C57BL/6 mice (Wu et al., 2010). In male Kunming mice, alteration of the light-dark schedule reduced total leukocyte and lymphocyte counts in the peripheral blood, as well as survival after transplantation with Ehrlich carcinoma cells (Li & Xu, 1997). In C57BL/6 mice, exposure to dim light during the period of darkness before wounding impaired healing and significantly delayed the time to wound closure compared with control mice and with mice housed under dim light conditions only after wounding (Walker et al., 2019).

In a study of cell-mediated and innate immune responses in male Siberian hamsters, exposure to dim light during the period of darkness reduced cell-mediated immune responses after sensitization and challenge with DNFB (Bedrosian et al., 2011). After challenge with LPS, bactericidal activity in plasma was also suppressed in hamsters exposed to dim light during the period of darkness compared with controls. Ikeno et al. (2014) also observed differences in response to LPS associated with an altered light–dark schedule in male Siberian hamsters. There was a significant increase in body temperature in all animals 1 hour after LPS injection; however, compared with controls, the febrile spike was significantly higher in animals housed under a shortened period of light. In contrast, Prendergast et al. (2015) reported that alterations in the light–dark schedule significantly reduced febrile responses in female Siberian hamsters after LPS treatment. Using a similar study design, Cable et al. (2017) found that punch biopsy wounds healed more slowly in female Siberian hamsters exposed to alterations in the light–dark schedule however, the timing of wounding did not affect healing.

Similar to the Bedrosian et al. (2011) study, Aubrecht et al. (2014) found that dim light during the period of darkness impaired DTH response to DNFB in combination with a shortened period of light in male Siberian hamsters. When the hamsters underwent 2 weeks of restraint stress in addition to alterations in the light–dark schedule, suppression was exacerbated compared with animals undergoing either restraint stress or dim light during the period of darkness alone.

4.1.3 Induces chronic inflammation

(a) Humans

Several studies evaluating the potential inflammatory effects of shift work were identified and are summarized in Table 4.6 and Table 4.7. Leukocyte count was the most commonly evaluated marker across studies (nine studies), followed by various cytokines, including IL6, C-reactive protein (CRP), and TNFα. [The Working Group noted that results were mixed across studies evaluating leukocyte count.] The largest of the studies was a cross-sectional evaluation of NHANES participants (Buss et al., 2018). No statistically significant differences in leukocyte count were observed when comparing day shift workers with night shift or rotating shift workers. [The Working Group noted that the limitations of the study included a lack of detail on shift schedules, single time-point of blood collection and a lack of specificity with regard to the time of day the blood samples were collected. The timing of blood sample collection is an important consideration as certain markers of inflammation exhibit diurnal variation, and

Table 4.6 End-points relevant to chronic inflammation in cross-sectional studies in shift workers

End-point[a]	Location, setting	Exposure level, and number of exposed and controls	Response (significance)	Covariates controlled	Reference
Leukocyte count	USA NHANES	6667 DS 970 NS/ES; 809 RS	–	Age, race, education, marital status, diabetes, smoking status, cold illness, waist circumference, HDL, total cholesterol, systolic blood pressure, BMI	Buss et al. (2018)
Leukocyte count	Japan Wholesale market	20 NS, 19 DS	–	None	Nakano et al. (1982)
Leukocyte count	Japan Iron-manufacturing company	20 RS (DS 07:00–15:00; AS 15:00–22:00; NS 22:00–07:00), 20 DS	–	None	Nakano et al. (1982)
Leukocyte count	Japan Male workers in synthetic-fibre manufacturing	107 DS, 101 RS (DS 07:00–14:00; AS 14:00–22:00; NS 22:00–07:00)	RS > DS ($P = 0.003$)	None	Nishitani & Sakakibara (2007)
Leukocyte count	Argentina Male factory workers	877 DS, 474 RS (28-day rotation: 4 DS 06:00 start; 3 rest days; 2 NS 18:00 start; 3 rest days; 4 NS 3 rest days; 2 DS; 3 rest days; 4 DS)	RS > DS ($P = 0.008$)	Age, metabolic syndrome	Sookoian et al. (2007)
Leukocyte count	Japan Male and female physicians at critical-care emergency centres and hospitals	27 RS (8–12 h including NS), 39 traditional shift (includes NS), 8 DS	–	None	Okamoto et al. (2008)
Leukocyte count	Finland Male and female airline workers	300 DS, 334 FS; 443 2-shift RS (DS and ES), 270 3-shift RS (DS, ES and NS), 530 in-flight[b]	Male 2-shift > DS ($P = 0.005$); male 3-shift > DS ($P = 0.021$); female in-flight < DS ($P = 0.005$)	Age, recent infectious disease, CRP levels, alcohol consumption, physical activity, education, smoking, obesity (BMI > 30), insomnia, sleep depth (h), perceived stress; co-exposure to ionizing radiation not considered	Puttonen et al. (2011)
Leukocyte count	Finland Male maintenance-unit airline workers	319 RS (2-shift DS and ES; 3-shift fast rotation with 1 DS, 1 ES and 1 NS; 3-shift slow rotation with 3 ES, 3 DS and 3 NS; flexible 3-shift with varied durations and consecutive numbers of shifts), 453 DS	–	Age, interaction between age and shift system	Viitasalo et al. (2015)

Table 4.6 (continued)

End-point[a]	Location, setting	Exposure level, and number of exposed and controls	Response (significance)	Covariates controlled	Reference
Leukocyte count	Republic of Korea Male workers in display manufacturing company	86 RS (6 NS, 2 rest days, 6 ES, 2 rest days, 6 DS, 2 rest days; mean duration, 10 yr), 46 former RS (mean duration, 2.79 yr), 112 DS	RS > DS ($P < 0.001$); former RS > DS ($P = 0.004$)	Age, BMI, alcohol consumption, smoking, regular exercise, sleep duration, sleep depth, sleep insufficiency, education, weekly work hours Multiple eligibility criteria	Kim et al. (2016)
Leukocyte count	Italy Female and male hospital nurses	84 DS, 71 NS	Monocyte count: NS > DS ($P < 0.001$)	Age, sex, BMI, WC, pack-years, binge drinking, CVD history, CRP, eosinophils, basophils, monocytes	Pavanello et al. (2017)
Cytokines	Japan Males and females nested in occupational cohort	3660 DS, 181 SW without NS, 1276 SW with NS, 142 NS	IL6: NS > DS ($P < 0.01$)	Age, sex, years of education, hours worked per week, annual household income, BMI, laboratory data (LDL-C and HbA1c), job stress Multiple eligibility criteria	Amano et al. (2018)
Cytokines	USA Males and females	11 DS (07:00–19:00, ≥ 3 shifts/wk for > 3 mo), 11 NS (19:00–07:00, ≥ 3 shifts/wk for > 3 mo)	NS > DS IL6 mesor ($P < 0.02$) and amplitude ($P < 0.02$)	Multiple eligibility criteria	Swanson et al. (2016)
Cytokines	Brazil Male workers in sanitary metals industry	21 DS (07:00–17:00) for median 4 yr, 17 NS (21:00–06:00) for median 3 yr	–	None	Reinhardt et al. (2019)
Cytokines	Brazil Male steel industry workers	9 NS (22:00–06:00), 6 EMS (06:00–14:00), and 7 DS (08:00–17:00); all schedules fixed for 2 yr	TNFα: EMS and NS > DS ($P < 0.0001$)	Multiple eligibility criteria	Crispim et al. (2012)
Cytokines	Germany Male and female industrial workers	137 DS, 225 SW (91% in 3-shift RS with NS, 9% in other including permanent NS)	–	None	van Mark et al. (2010)
Cytokines	Italy Hospital nurses	28 DS, 68 RS (1 day shift, 06:00–14:00; 1 AS 14:00–22:00; 1 NS 22:00–06:00); ≥ 2 yr in current schedule	TNFα and IL1β: RS < DS ($P \leq 0.03$)	Job seniority, and presence of offspring	Copertaro et al. (2011)
Cytokines	Finland Male and female airline workers	300 DS, 334 FS, 443 2-shift RS (DS and ES), 270 3-shift RS (MS, ES and NS), 530 in-flight[b]	CRP: male 3-shift > DS ($P = 0.002$); female 2-shift > DS ($P = 0.03$)	Age, recent infection or disease, CRP levels, alcohol consumption, physical activity, education, smoking, obesity (BMI > 30), insomnia, sleep depth (h), perceived stress	Puttonen et al. (2011)

Table 4.6 (continued)

End-point[a]	Location, setting	Exposure level, and number of exposed and controls	Response (significance)	Covariates controlled	Reference
Cytokines	Belgium Male steel workers	9 DS (mean, 13 yr), 16 fast clockwise RS (mean, 14 yr), 18 slow counter-clockwise RS (mean, 16 yr) (RS: DS 06:00–14:00; AS 14:00–22:00; NS 22:00–06:00)	–	None	Kantermann et al. (2014)
Cytokines	Finland Male maintenance-unit airline workers	319 RS (2-shift DS and ES; 3-shift fast rotation with 1 DS, 1 ES and 1 NS; 3-shift slow rotation with 3 ES, 3 DS and 3 NS; flexible 3-shift rotation with varied durations and consecutive numbers of shifts), 453 DS	–	Minimal adjustment for covariates	Viitasalo et al. (2015)
Cytokines	Republic of Korea Male workers in display manufacturing company	86 RS (6 NS, 2 rest days, 6 ES, 2 rest days, 6 DS, 2 rest days; mean duration, 10 yr), 46 former RS (mean duration, 2.79 yr), 112 DS	CRP: RS > DS ($P = 0.002$)	Age, BMI, alcohol consumption, smoking, regular exercise, sleep duration, sleep depth, sleep insufficiency, education, weekly work hours Multiple eligibility criteria	Kim et al. (2016)
Cytokines	Italy Female and male hospital nurses	84 DS, 71 NS	CRP: NS > DS ($P < 0.001$)	Age, sex, BMI, WC, pack-years, binge drinking, CVD history, CRP, eosinophils, basophils, monocytes	Pavanello et al. (2017)
LPS-binding protein	USA Males and females	11 DS (07:00–19:00, ≥ 3 shifts/wk for > 3 mo); 11 NS (19:00–07:00, ≥ 3 shifts/wk for > 3 mo)	LPS mesor: NS > DS ($P < 0.01$)	Multiple eligibility criteria	Swanson et al. (2016)

–, not significant; AS, afternoon shift; BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular disease; DS, day shift; EMS, early morning shift; ES, evening shift; FS, former shift worker; h, hour; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; IL, interleukin; LDL-C, low-density lipoprotein-cholesterol; LPS, lipopolysaccharide; mo, month; MS, morning shift; NHANES, National Health and Nutrition Examination Survey; NS, night shift; RS, rotating shift; SW, shift work; TNFα, tumour necrosis factor alpha; WC, waist circumference; wk, week; yr, year.

[a] End-points measured in blood, except Reinhardt et al. (2019) (measured in saliva).

[b] Irregular working hours with MS, ES, and NS of different length, and with or without time lag.

Table 4.7 End-points relevant to inflammation in non-cross-sectional studies of shift work

End-point[a]	Location, setting	Exposure level, and number of exposed and controls	Response (significance)	Covariates controlled	Reference
Leukocyte count	Islamic Republic of Iran NR	25 randomized to 3 DS, 1 rest day, and 3 NS; 25 randomized to 3 NS, 1 rest day, and 3 DS	NS > DS ($P < 0.01$)	Multiple eligibility criteria; within-person analysis	Khosro et al. (2011)
Platelets	Islamic Republic of Iran NR	25 randomized to 3 DS, 1 rest day, and 3 NS; 25 randomized to 3 NS, 1 rest day, and 3 DS	NS > DS ($P < 0.01$)	Multiple eligibility criteria; within-person analysis	Khosro et al. (2011)
Cytokines	Islamic Republic of Iran NR	25 randomized to 3 DS, 1 rest day, and 3 NS; 25 randomized to 3 NS, 1 rest day, and 3 DS	NS > DS for IL6 and CRP ($P < 0.01$)	Multiple eligibility criteria; within-person analysis	Khosro et al. (2011)
Cytokines	Canada Healthy men and women in laboratory study	8 participants transitioned from DS to NS	NS vs DS phase advance in IL6, TNFα, and IL1β secretion/monocyte ($P < 0.01$)	Multiple eligibility criteria; within-person analysis	Cuesta et al. (2016)

CRP, C-reactive protein; DS, day shift; IL, interleukin; NR, not reported; NS, night shift; RS, rotating shift; TNFα, tumour necrosis factor alpha; vs, versus.

[a] All end-points measured in blood.

leukocyte count has been shown to vary diurnally (Sennels et al., 2011).] Other cross-sectional studies were reviewed (Nakano et al., 1982; Nishitani & Sakakibara, 2007; Sookoian et al., 2007; Okamoto et al., 2008; Puttonen et al., 2011; Viitasalo et al., 2015; Kim et al., 2016; Pavanello et al., 2017). [The Working Group noted that limitations across these studies included small sample sizes, single time-points of blood collection, limited or no adjustment for covariates, and poorly defined shift schedules, including a lack of data on the duration that participants worked in the various shift schedules.] Two of the studies did not collect blood samples during or immediately after night shift work (Puttonen et al., 2011; Viitasalo et al., 2015), and two of the studies did not specify when samples were collected relative to completion of night shift work (Nishitani & Sakakibara, 2007; Sookoian et al., 2007).

In the only study of leukocyte count that was not of cross-sectional design, 50 participants were randomized to either start with 3 day shifts and then transition to 3 night shifts, or start with 3 night shifts and then transition to 3 day shifts. Blood samples were collected in the morning during both day shift and night shift periods. Night shift work was associated with an elevated leukocyte count (Khosro et al., 2011).

Results of the studies evaluating IL6, CRP, and TNFα were mixed. The majority of studies were cross-sectional in design, the largest of which included over 5000 participants from an occupational cohort in Japan (Amano et al., 2018). Night shift workers were found to have statistically significantly higher circulating levels of IL6 compared with day shift workers. No significant differences in CRP were observed. In addition to a lack of detail with regard to shift schedules, blood samples were collected at single time-points that were not specified. [The Working Group noted that this is a concern for IL6, which has been observed to vary rhythmically over a 24-hour day (Bogaty et al., 2013; Nilsonne et al., 2016); however, it may not be a limitation when considering CRP, for which levels in blood seem to be fairly stable over time (Meier-Ewert et al., 2001, 2004).] Although a few studies did capture cytokine measurements at multiple specified time-points, two of the studies were small in size and did not adjust for potential confounding factors (Swanson et al., 2016; Reinhardt et al., 2019). Also, in one of the studies that restricted enrolment to healthy, non-obese men who did not use medications, alcohol, or tobacco, the levels for each cytokine were simply averaged over the multiple time periods, meaning that time period-specific effects were not considered (Crispim et al., 2012). Many of the cross-sectional studies evaluating cytokines did not collect blood samples during or immediately after night shift work (or blood sample timing relative to night shift work was unspecified) (van Mark et al., 2010; Copertaro et al., 2011; Puttonen et al., 2011; Kantermann et al., 2014; Viitasalo et al., 2015; Kim et al., 2016). [The Working Group noted that, of these studies, those reporting null associations may have failed to capture the more acute inflammatory effects of night shift work that might have dissipated over subsequent days during which night shift work was not conducted.]

Only two non-cross-sectional studies of cytokines were identified, and both evaluated relatively short periods of exposure to night shift work (see Table 4.7). In the randomized trial of night shift work (either starting with 3 day shifts and then transitioning to 3 night shifts, or starting with 3 night shifts and then transitioning to 3 day shifts), statistically significantly elevated circulating levels of IL6 ($P < 0.01$) and CRP ($P = 0.01$), but not TNFα, were observed in association with night shift work (Khosro et al., 2011). In a laboratory-based study of eight healthy participants, it was found that transitioning from a day shift schedule to a night shift schedule resulted in statistically significant phase advances in the per-cell secretion of IL1β,

IL6, and TNFα, in stimulated monocytes (Cuesta et al., 2016).

(b) Experimental systems

See Table 4.8.

Alteration in the light–dark schedule has been shown to enhance inflammation in rodent studies and models of inflammatory disease. Polidarová et al. (2017) examined the expression levels of pro-inflammatory cytokine genes in colonic mucosa obtained from Wistar rats exposed to shifts in the light–dark schedule. In rats for which the light–dark schedule was advanced and then delayed, there were minimal changes in expression of pro-inflammatory genes; however, expression of regulator of G protein signalling 16 (*Rsg16*), which regulates the inflammatory response, was suppressed. Exposure to continuous light significantly upregulated the expression of *IL1α*, IL17 receptor α, and signal transducer and activator of transcription 3 (*Stat3*), but had no effect on *TNFα* or *Rsg16* gene expression in the colon (Polidarová et al., 2017). Guerrero-Vargas et al. (2017) investigated the influence of alteration in the light-dark schedule on tumour growth (see Table 3.5) and inflammation in male Wistar rats. Plasma TNFα levels were significantly higher in rats exposed to continuous light at both 40 and 80 minutes after a single injection of LPS.

In a study of the impact of alteration of the light–dark schedule on the development and progression of colitis, extensive tissue destruction and mucosal ulceration was seen in C57BL/6 mice given 2% dextran sodium sulfate (DSS) in drinking-water and exposed to advances in the light-dark schedule (Preuss et al., 2008). In mice given DSS without alteration of the light-dark schedule, only a mild infiltration of inflammatory cells and reduction in the number of goblet cells in the mucosa were observed. Myeloperoxidase activity was also significantly higher with alteration of the light-dark schedule in mice given DSS (Preuss et al., 2008). Summa et al. (2013) examined the effects of alteration of the light–dark schedule on alcohol-induced liver and gastrointestinal tract inflammation in C57BL/6J mice. Advances in the light–dark schedule resulted in a significant increase in alcohol-induced intestinal permeability, an elevation of serum LPS, and hepatic steatosis compared with mice on the same diet that were not subject to alteration of the light-dark schedule. Alteration of the light–dark schedule alone increased intestinal permeability to a similar degree as observed in mice fed alcohol in the absence of light–dark schedule alterations. Increased levels of mRNA for the inflammatory mediators *IL1β* and *IL6* were observed in liver tissue from male C57BL/6 mice exposed to an altered light–dark schedule, which could be modulated by a nutrient-rich dietary supplement in the form of essence of chicken (Wu et al., 2015). Khalyfa et al. (2017) examined the inflammatory status of visceral white adipose tissue in male C57BL/6 mice. Total macrophage numbers, as well as the number of pro-inflammatory M1 macrophages, were significantly increased, while the number of FoxP3+ cells (regulatory T lymphocytes) was decreased, in mice exposed to an inverted light–dark schedule. Hand et al. (2016) showed that continuous light disrupted the night-time repression of inflammatory pathways in male DBA/1 mice immunized with collagen in an established model of inflammatory arthritis. Serum cytokine levels and joint swelling demonstrated diurnal variation in arthritic mice, with serum levels of several pro-inflammatory cytokines showing significant elevation during the period of light and a time-dependent fluctuation in disease severity. In arthritic mice exposed to continuous light, diurnal variation in paw size was absent and pro-inflammatory cytokine expression remained elevated in inflamed joints throughout a 24-hour period, although there were no differences in overall disease incidence or severity. Male CD1 mice exposed to continuous light demonstrated elevated levels of granulocytic and monocytic

Table 4.8 Inflammation in response to alterations in the light–dark schedule in experimental systems

Experimental system	Exposure	Relevant finding(s)	Reference
Wistar rat (M, F)	Control: LD12:12 Treatment: continuous light for 4 wk, or shift in the light-dark schedule (6-h advance for 2 d followed by 6-h delay for 2 d, with 4 advances and 4 delays over 16 d)	Continuous light: ↑ expression of pro-inflammatory cytokine genes (*IL1α*, *IL17rα*, *Stat3*) Shift in the light-dark schedule: suppression of the immunoregulatory gene *Rgs16* in colonic tissue	Polidarová et al. (2017)
Wistar rat (M)	Control: LD12:12 Treatment: continuous light for 5 wk Intravenous injection of LPS at 2 µg/kg bw 1 wk after removal from continuous light	↑ Plasma TNFα (40 and 80 min) after LPS injection	Guerrero-Vargas et al. (2017)
C57BL/6 mouse (M)	Control: LD12:12 Treatment: advance in the light-dark schedule by 12 h every 5 d for 3 mo 2% dextran sodium sulfate beginning after the 19th advance in the light-dark schedule	↑ Inflammation, tissue damage, and myeloperoxidase activity in the colon	Preuss et al. (2008)
C57BL/6 mouse (M)	Control: LD12:12 Treatment: 12-h advance in the light-dark schedule 1×/wk for 22 wk Chronic alcohol diet beginning 3 mo after the onset of shifts in the light-dark schedule	↑ Intestinal permeability (advance in the light-dark schedule without alcohol) ↑ Alcohol-induced intestinal permeability, elevation of serum LPS, and hepatic steatosis	Summa et al. (2013)
C57BL/6 mouse (M)	Control: LD12:12 Treatment: reversed the light-dark schedule every 4 d for 12 wk	↑ mRNA for *IL-1β* and *IL-6* in liver	Wu et al. (2015)
C57BL/6 mouse (M)	Control: NR Treatment: reversed the light-dark schedule every 2 wk for 8 wk	↑ Numbers of M1 macrophages in adipose tissue ↓ Number of FoxP3+ cells (regulatory T lymphocytes)	Khalyfa et al. (2017)
DBA/1 mouse (M)	Control: LD12:12 Treatment: continuous light 1 mg/mL bovine type II collagen emulsified in CFA injected intradermally at the base of the tail after 2 wk of exposure to continuous light	Altered diurnal variation in paw size and expression of pro-inflammatory cytokines	Hand et al. (2016)
CD1 mouse (M)	Control: LD12:12 Treatment: continuous light Heat-inactivated *Mycobacterium tuberculosis* emulsified in CFA injected into the footpad before exposure to continuous light	↑ Numbers of MDSC ↓ Number of circulating T lymphocytes (both $CD4^+$ and $CD8^+$)	Perfilyeva et al. (2017)
Swiss Webster mouse (M)	Control: LD14:10 Treatment: LD14:10 with dim light (5 lux) during the period of darkness for approximately 4 wk Injection of LPS (0.5 mg/kg bw) after 4 wk of alteration in the light-dark schedule	↑ Expression of pro-inflammatory genes (*TNFα*, *IL1β*, and *IL6*) in microglial cells	Fonken et al. (2013)

Table 4.8 (continued)

Experimental system	Exposure	Relevant finding(s)	Reference
Per2^{Luc} knock-in mouse	Control: LD12:12 Treatments: advance in the light-dark schedule (18:6, 1×/wk for 4 wk)	Hypothermia and reduced survival after injection of LPS (12.5 mg/kg bw) ↑ Serum levels of IL1β, GM-CSF, IL12, and IL13	Castanon-Cervantes et al. (2010) (see also Section 4.2.2)
Per2^{Luc} knock-in mouse	Control: LD12:12 Treatments: advances in the light-dark schedule (18:6, 1×/wk for 4 wk) Intraperitoneal injection of LPS (5 mg/kg bw) 7 d after final advance in the light–dark schedule	↑ Serum levels of IL6, IL18, MIP-2, and LIF-2	
Per2^{Luc} knock-in mouse	Control: LD12:12 Treatments: advances in the light-dark schedule (18:6, 1×/wk for 4 wk)	↑ Secretion of IL6 from peritoneal macrophages cultured with LPS (10 μg/mL) ex vivo	
Per2^{Luc} knock-in mouse	Control: LD12:12 Treatments: advances in the light-dark schedule (18:6, 1×/wk for 12 wk)	↑ Peripheral blood levels of IL6 after culture with LPS (50 μg/mL) ex vivo	Brager et al. (2013)

↑, increase; ↓, decrease; bw, body weight; CFA, complete Freund adjuvant; d, day; F, female; GM-CSF, granulocyte macrophage colony-stimulating factor; h, hour; IL, interleukin; LD, light–dark schedule, light(h):darkness(h); LIF-2, leukaemia inhibitory factor-2; LPS, lipopolysaccharide; M, male; MDSC, monocytic myeloid-derived suppressor cells; MIP-2, macrophage inflammatory protein-2; mo, month; NR, not reported; Rgs16, regulator of G protein signalling 16; Stat3, signal transducer and activator of transcription 3; TNFα, tumour necrosis factor alpha; wk, week.

myeloid-derived suppressor cells (MDSCs) (Perfilyeva et al., 2017). The increase was greater in the presence of adjuvant-induced arthritis; however, there was no change in inflammatory measures of disease such as paw thickness. At 4 weeks, the changes in the number of MDSCs were accompanied by a corresponding significant decrease in the numbers of CD4+ and CD8+ T lymphocytes. Continuous exposure to dim light increased the expression of pro-inflammatory genes in microglial cells obtained from male Swiss Webster mice after LPS administration (Fonken et al., 2013). In *Per2*^{Luc} mice maintained on a high-fat diet (60% fat calories, 20% protein calories, 20% carbohydrate calories), multiple shifts in the light–dark schedule resulted in an elevation of the total number of mature macrophages and the overall percentage of pro-inflammatory M1 macrophages in adipose tissue (Kim et al., 2018) (see Section 4.2.2(b)). A corresponding significant decrease in the percentage of anti-inflammatory M2 macrophages was also noted. Bone marrow-derived macrophages cultured from cells obtained from mice exposed to shifts in the light–dark schedule showed similar elevations in the percentage of M1 macrophages to that from cells of control mice. mRNA expression of the pro-inflammatory cytokines *IL1β*, *IL6*, and *TNFα* was elevated 2–3-fold in cultured adipose tissues from mice exposed to an altered light–dark schedule relative to controls, and was elevated 3–5-fold in bone marrow-derived macrophages after in vitro stimulation with LPS relative to cells from control mice (Kim et al., 2018; see Section 4.2.2(b)). Castanon-Cervantes et al. (2010) examined the immune response to LPS in *Per2*^{Luc} knock-in mice. Mice exposed to an altered light–dark schedule showed significant and persistent hypothermia after intraperitoneal challenge with LPS, and survival was significantly reduced in mice exposed to 4 weeks of advances in the light–dark schedule. Serum levels of pro-inflammatory mediators were significantly elevated in mice that were exposed to light–dark schedule shifts and challenged with LPS. Peritoneal macrophages obtained from mice exposed to advances in the light–dark schedule also demonstrated elevated IL6 secretion when cultured in vitro with LPS (Castanon-Cervantes et al., 2010) (see also Section 4.2.2(b)). IL6 levels were also significantly elevated after ex vivo LPS challenge in peripheral blood collected from *Per2*^{Luc} knock-in mice that had been exposed to advances in the light–dark schedule (Brager et al., 2013).

4.1.4 Is genotoxic

(a) Humans

No data were available to the Working Group on genotoxicity, other than in aircrews. Aircrews that engage in transmeridian travel have jobs with considerable circadian disruption. Studies on the key characteristics of carcinogens in aircrews have been conducted, but the focus of these studies was on associations with ionizing radiation exposure, with little to no consideration of the potential contribution of circadian disruption. For example, multiple studies in aircrews have reported on chromosomal damage (Romano et al., 1997; Cavallo et al., 2002a; Bolzán et al., 2008), sister-chromatid exchanges (Silva et al., 1999), gaps, breaks, and translocations (Cavallo et al., 2002b), dicentric formation (De Luca et al., 2009), and translocations (in pilots with flight years, Yong et al., 2009; with commercial flight cosmic radiation exposure, Grajewski et al., 2018). Only the study by Grajewski et al. (2018) considered the role of circadian disruption on the occurrence of chromosomal damage. However, because of the high degree of correlation between the measures of ionizing radiation exposure and cumulative time zones crossed (Pearson correlation coefficient, 0.89), independent associations could not be assessed. [In light of the well-established effects of ionizing radiation on many of the key characteristics of carcinogens, and the lack of data on the independent effects

of circadian disruption in aircrews, the Working Group concluded that mechanistic studies in aircrews were uninformative.]

(b) Experimental systems

No data were available to the Working Group on genotoxicity in experimental systems.

4.1.5 Induces epigenetic alterations

(a) Humans

See Table 4.9.

Methylation of clock genes is discussed in Section 4.2.2.

Alterations in DNA methylation patterns have been studied in night shift workers, with no effect of shift work on DNA methylation at Alu and long interspersed nuclear element-1 repetitive elements or on promoter methylation of glucocorticoid receptor, *IFNγ*, or *TNFα* genes (Bollati et al., 2010). [The Working Group noted that there was only one time-point at which blood was collected, and the timing of this collection was not specified.]

Four reports from the same group evaluated the effects of long-term night shift work (> 10 years) (Zhu et al., 2011; Jacobs et al., 2013; Shi et al., 2013; Liu et al., 2015). A genome-wide methylation analysis revealed widespread methylation alterations in night shift workers at many methylation- and cancer-relevant genes (Zhu et al., 2011). Forty-eight CpG loci, corresponding to 29 microRNAs (miRNAs), were methylated differently in night shift workers (categorized into three groups according to duration of night shift work) than in day shift workers, including the circadian-relevant miR219, with expression implicated in several cancers (Shi et al., 2013). Long-term night shift work altered the methylation patterns at imprinted genes, such as *DLX5*, *IGF2-AS*, and *TP73* (Jacobs et al., 2013). Long-term night shift work (> 10 years) was associated with the methylation-based suppression of *miR34b*, which is a known tumour suppressor (Liu et al., 2015). [The Working Group noted that the limitations of these studies included the small sample of long-term shift workers, the lack of detail on intensity and frequency of shift work, and the lack of specificity of timing of the blood sample collection. Diurnal variation of lymphocyte subsets may lead to biased DNA methylation measurements. Adjusting for lymphocyte cell profile, which the studies did not do, could have addressed this issue.]

Assays for genome-wide DNA methylation in blood samples revealed that different loci were affected between night and day shift workers, and the average methylation was decreased in night shift workers (working ≥ 24 hours per week and ending no earlier than 06:00 for 6 months) across 16 135 CpG loci (Bhatti et al., 2015). Adams et al. (2017) reported no statistically significant associations at the genome-wide level with shift work or chronotype; the timing of the single sample collection was not specified, but adjustment for leukocyte cell profile was performed.

A cross-sectional study in Lodz, Poland, reported that current rotating shift work was not associated with methylation status of promoter sites within the *BRCA1* and *BRCA2* genes (Peplonska et al., 2017).

(b) Experimental systems

See Table 4.10.

Several studies have reported that DNA methylation may be influenced by induction or repression of DNA methylation enzymes caused by alterations in the light–dark schedule. In particular, DNA methyl transferase-1 protein levels increased by more than 2-fold in the liver of female Sprague-Dawley rats exposed to repeated advances of the light–dark schedule (Kochan et al., 2015). In ovariectomized athymic, inbred nude Crl:NIH-*Foxn1*^{rnu} rats, dim light during the period of darkness increased *Stat3* expression via an epigenetic mechanism, of Aplasia Ras homologue member I (ARHI) promoter methylation (Xiang et al., 2019).

Table 4.9 DNA methylation in the blood of night shift workers

Location, setting, study design	Exposure level, and number of exposed and controls	Response (significance)	Covariates controlled	Reference
Italy Chemical plants Cross-sectional study	150: 100 RS (MS, 06:00–14:00; AS, 14:00–22:00; NS, 22:00–06:00; worked 4 d followed by 2 rest days), 50 DS	– (Alu and LINE-1 elements; *GCR*, *TNFα*, *IFNγ* genes)	Age, BMI, job seniority	Bollati et al. (2010)
Denmark[a]	29: 19 long-term NS (≥ 10 yr of work starting at 19:00 or later and ending before 09:00), 10 DS	+ (Genome-wide methylation; Illumina Infinium Methylation Chip)	Age, folate intake	Zhu et al. (2011)
Denmark[a]	20: 10 long-term NS (≥ 10 yr of work starting at 19:00 or later and ending before 09:00), 10 DS	48 CpG of 29 miRNAs: hypermethylation, NS > DS 2 CpG of 2 miRNAs: hypomethylation, NS > DS (Genome-wide methylation; Illumina Infinium Methylation Chip)	Age, folate intake	Shi et al. (2013)
Denmark[a]	20: 10 long-term NS (≥ 10 yr of work starting at 19:00 or later and ending before 09:00), 10 DS	397 CpG in 56 imprinted genes: 20 hypermethylation ($P < 0.05$), 30 hypomethylation ($P < 0.001$) in NS (Genome-wide methylation; Illumina Infinium Methylation Chip)	Age, folate intake	Jacobs et al. (2013)
Denmark[a]	20: 10 long-term NS (≥ 10 yr of work starting at 19:00 or later and ending before 09:00), 10 DS	*miR34b* hypermethylation: NS > DS ($P = 0.016$) (Genome-wide methylation; Illumina Infinium Methylation Chip)	Age, folate intake	Liu et al. (2015)
USA Health-care workers Cross-sectional study	124: 59 NS, 65 DS Blood collected within 2 h of completing the work shift on the 3rd day (07:00–09:00 for NS and 17:00–19:00 for DS)	NS < DS (FDR: $q < 0.05$) (Genome-wide methylation; Illumina Infinium Methylation Chip)	Age, sex, BMI, race, smoking status, leukocyte cell profile (by flow cytometry)	Bhatti et al. (2015)
USA Health-care workers Cross-sectional study	111 NS, 86 DS (female) 110 female NS, 131 male NS	– (Genome-wide alteration; Illumina Infinium Methylation Chip)	Age, BMI, race, alcohol consumption, smoking, leukocyte cell mixture	Adams et al. (2017)

Table 4.9 (continued)

Location, setting, study design	Exposure level, and number of exposed and controls	Response (significance)	Covariates controlled	Reference
Poland Nurses and midwives Cross-sectional study	710: 347 rotating NS (night, 19:00–07:00; day, 07:00–19:00) and 363 DS (07:00–16:00) Blood collected 06:00–10:00	– *BRCA1* and *BRCA2* gene promoters	Night work status, smoking, obesity, physical activity, alcohol consumption	Peplonska et al. (2017)

–, not significant; +, significant; AS, afternoon shift; BMI, body mass index; CpG, cytosine–phosphate–guanine; d, day; DS, day shift; FDR, false discovery rate; GCR, glucocorticoid receptor; h, hour; IFN, interferon; LINE-1, long interspersed nuclear element-1; MS, morning shift; NS, night shift; RS, rotating shift; TNFα, tumour necrosis factor alpha.

[a] Study participants had previously participated in the Danish Diet, Cancer and Health prospective cohort.

Table 4.10 Epigenetic alterations in response to alterations in the light–dark schedule in experimental systems

Experimental system	Exposure	Relevant finding(s)	Reference
Sprague-Dawley rat (F)	Control: LD12:12 Treatments: advance in the light–dark schedule by 3 h each day for 6 d; advance in the light–dark schedule by 3 h each day for 6 d followed by a 10-d regular LD12:12 for a total of 54 d (6–10–6–10–6–10–6)	↑ (by > 2-fold) in DNMT1 protein levels in the liver samples (6–10–6–10–6–10–6) ↓ miR127 and miR146b levels and activity in mammary tissues (both treatments)	Kochan et al. (2015)
Ovariectomized athymic, inbred nude Crl:NIH-*Foxn1*^{rnu} rat (F)	Control: LD12:12 Treatment: LD12:12, with dim light during period of darkness; human MCF-7 tumour implantation 1 wk after start of exposure to dim light	↑ *Stat3* expression via ARHI promotor methylation	Xiang et al. (2019)
Adult C57BL/6J mouse (M)	Mice were housed in two different light–dark schedules with light during 06:00–18:00 or 18:00–06:00 Treatments: advance in the light–dark schedule by 6 h by turning on the lights at ZT18; advance in the light–dark schedule by 6 h by turning on the lights at ZT18 1×/wk for 8 wk Analysis 1 wk after the final advance; livers collected around the clock	Rapid H3K4me3 histone modification (trimethylation of lysine 4 on histone 3) in livers at four time-points (ZT3, ZT8, ZT15, and ZT20) (both treatments)	Grygoryev et al. (2018)
C3H/HePas mouse (M)	Control: LD12:12 Treatment: continuous light for 8 wk	↑ Expression of *Rev-erbα*-targeting *miR140–5p*, *185–5p*, *326–5p*, and *328–5p* in liver	Borck et al. (2018)
Golden Syrian hamster (M)	Control: LD12:12 Treatments: abrupt shift to LD8:16 or to LD16:8 for 3 wk; continuous dim light (30 lux) for 3 wk, followed by continuous bright light (100 lux) for 3 wk	Altered expression of *miR132*, *miR212*, and their direct target, methyl CpG-binding protein *MeCP2*, in SCN tissues (both treatments)	Mendoza-Viveros et al. (2017)

↑, increase; ↓, decrease; ARHI, aplasia Ras homologue member I; d, day; DNMT1, DNA methyltransferase-1; F female; h, hour; H3K4me3, trimethylation of lysine 4 on histone 3; LD, light–dark schedule, light(h):darkness(h); miR, microRNA; *Rev-erb,* circadian nuclear receptor gene; SCN, suprachiasmatic nucleus; STAT3, signal transducer and activator of transcription-3; wk, week; ZT, Zeitgeber time.

Epigenetic regulation via histone modifications was associated with both single and repeated advances in the light–dark schedule (Grygoryev et al., 2018). In particular, trimethylation of lysine 4 on histone 3 (H3K4me3) circadian histone modification in the liver of mice responded rapidly to an altered light–dark schedule. Epigenetic regulation via miRNA mechanism was also influenced by alteration in the light-dark schedule. For example, the expression of *Rev-erbα*-targeting miRNAs, *miR140–5p*, *185–5p*, *326–5p*, and *328–5p*, was increased in liver cells of C3H/HePas mice exposed to continuous light (Borck et al., 2018). Changes in *miR127* and *miR146b* levels in mammary tissues of female Sprague-Dawley rats were induced by both short-term and repeated shifts in the light–dark schedule (Kochan et al., 2015). In the suprachiasmatic nucleus tissues of male golden Syrian hamsters, advances in the light–dark schedule influenced expression of *miR132* and *miR212*, and of their direct target, methyl CpG-binding protein (*MeCP2*) (Mendoza-Viveros et al., 2017).

Studies on telomere shortening in experimental systems are addressed in Section 4.1.8(b).

4.1.6 *Modulates receptor-mediated effects: endocrine hormones*

(a) Humans

See Table 4.11.

(i) Estrogens and progesterone

In a cross-sectional analysis, there was an increase in plasma estradiol (but not estrone) with duration of shift work in postmenopausal women who participated in the Nurses' Health Studies (NHS-I and NHS-II) (Schernhammer et al., 2004). In another cross-sectional study of 177 Japanese postmenopausal women who were not using hormone replacement therapy, ever working at night was associated with elevated estrone serum levels, and estradiol levels were modestly elevated (Nagata et al., 2008). In the second Nurses' Health Study (NHS-II) cohort, there was a modest inverse association of 6-sulfatoxymelatonin (aMT6s) levels and follicular estradiol ($P = 0.07$) in 459 largely premenopausal women; no significant association was seen with estradiol, progesterone, estrone or estrone sulfate (or with dehydroepiandrostenedione, dehydroepiandrostenedione sulfate, testosterone, or androstenedione) measured either in the luteal or the follicular phase of the menstrual cycle (Schernhammer et al., 2006). In a study of 31 premenopausal Italian nurses engaged in a rapid forward rotating shift schedule (1 morning, 1 afternoon, 1 night, followed by 2 days off) maintained for at least the last 2 months, serum levels of 17-β-estradiol measured in the follicular phase of the menstrual cycle were elevated in shift work nurses compared with daytime nurses, particularly in those who did not take a nap during their shift relative to those who did (Bracci et al., 2013, 2014). [The Working Group noted that these findings suggest that circadian disruption from night shift work would affect estrogen level independently of melatonin suppression.] In a study of 94 premenopausal shift-working nurses, 82 of whom completed follow-up, an inverse relationship between aMTS6 and serum estradiol in winter was suggested, but the association lacked statistical significance when adjusted for multiple covariates including menstrual cycle stage (Langley et al., 2012). Similarly, covariate adjustment attenuated observed associations in increased levels of estradiol, estrone, and progesterone with increasing years of night shift work history. Sixty-three women engaged in a rapidly forward rotating night shift work schedule (2 or 4 mornings, 2 afternoons, 2 nights, and 2 days off) had higher serum estradiol and progesterone levels, especially in the follicular phase of the menstrual cycle, compared with 73 day shift workers (Gómez-Acebo et al., 2015). In another study in Spain in 75 workers on either a rapidly or slowly rotating night shift schedule and 42 daytime workers, levels of total progestagens

Table 4.11 Endocrine receptor-mediated effects in shift workers

End-point	Location, setting, study design	Exposure level, and number of exposed cases	Response (significance)	Covariates controlled	Reference
Estrogen	USA Nurses' Health Studies (NHS-I and NHS-II) Cross-sectional	> 3 yr rotating NS in 644 postmenopausal women (hormone replacement therapy not used within 3 mo of blood draw); number of NS worked in the last 2 wk in 79 premenopausal women (hormones or oral contraceptives not used in prior 6 mo)	Increase in estradiol ($P = 0.03$), but not estrone ($P = 0.34$), with duration of rotating NS in postmenopausal women; no association with recent NS in premenopausal women	Age, BMI, time of blood draw, laboratory batch	Schernhammer et al. (2004); see also Table 4.15
Estrogen, testosterone	Japan Breast cancer screening Cross-sectional	7 ever vs 170 never worked at night; postmenopausal women not using hormone replacement therapy	Ever > never, estrone ($P = 0.006$); modest increase in estradiol ($P = 0.11$), no effect on testosterone	Age, BMI, smoking status, alcohol consumption, group of participants, day length before urine collection	Nagata et al. (2008); see also Table 4.15
Estrogen, testosterone, progesterone, androstenedione, DHEA, DHEAS	USA Nurses' Health Study II (NHS-II) Cross-sectional	459 women (384 controls from a nested case–control study of urinary melatonin levels and breast cancer risk, 80 premenopausal women from a validation study), of whom 44 had ≥ 1 NS in the last 2 wk and 310 had ≥ 1 yr of rotating NS	Modest inverse association of follicular estradiol and aMT6s levels ($P = 0.07$) in premenopausal women – (for sex hormones measured either in the luteal or the follicular phase of the menstrual cycle)	Age, BMI, weight change from age 18 yr, alcohol consumption, month of urine collection, antidepressant use, aspirin use, physical exercise, smoking history and pack-years smoked, height, first spot morning urine, sleep duration	Schernhammer et al. (2006)
Estrogen	Italy Hospital nurses Cross-sectional	31 healthy premenopausal nurses (not using drug treatments) on rapid forward rotating NS in the last ≥ 2 mo; 31 DS nurses	NS who did not take naps during their shift > NS who did take naps or in DS workers (estradiol levels, $P < 0.05$)	Age, BMI, physical exercise, smoking	Bracci et al. (2013); see also Table 4.1
Estrogen	Italy Nurses Cross-sectional study	116 F nurses: 60 with ≥ 2 yr of SW, 56 permanent DS	NS > DS (17-β-estradiol levels, $P = 0.040$)	Age, physical activity, number of offspring	Bracci et al. (2014); see also Table 4.1
Estradiol, estrone, progesterone	Canada Shift-working nurses, F Cross-sectional	Years of NS work in 94 premenopausal nurses working a full-time RS schedule	–	Age, BMI, menstrual cycle stage, OC use, recent alcohol consumption, recent caffeine consumption, smoking status, recent physical activity	Langley et al. (2012)

Table 4.11 (continued)

End-point	Location, setting, study design	Exposure level, and number of exposed cases	Response (significance)	Covariates controlled	Reference
Estrogen, progesterone, testosterone	Spain Health-care workers and teachers Cross-sectional	63 F rotating NS, 73 F DS (no hormone replacement therapy, no OC use in last 6 mo)	Rotating NS > DS for all women (estradiol, $P = 0.041$; progesterone, $P = 0.008$) Rotating NS < DS for women in the luteal phase (testosterone, $P = 0.024$)	Menopausal status, season, age, BMI, number of cigarettes the day before	Gómez-Acebo et al. (2015)
Estrogen, progesterone, androgens	Spain Hospital, car industry, railway company Cross-sectional	75 rotating NS (rapid and slow), 42 DS, both sexes; 53 women, not using hormone therapy or OCs (16 each of NS and DS who were premenopausal; 4 DS and 17 NS who were postmenopausal)	NS > DS (progestagens, $P < 0.05$; androgen, $P < 0.05$) – (estrogen)	Age, sex, BMI, menopausal status, menstrual phase, education, smoking, physical activity, caffeine, sleep, parity, age at first full-term birth, chronic symptoms, drug use, hours of sunlight	Papantoniou et al. (2015)
Estrogen	Poland Hospital nurses and midwives Cross-sectional	263 F NS workers and 269 DS not using OCs or hormone replacement therapy (345 premenopausal, 187 postmenopausal); every NS was followed by a day off	– (for premenopausal women) Association in postmenopausal modified by chronotype with a significant increase with years of NS in morning chronotypes (P for trend, < 0.001) but not in evening chronotypes (P for trend, 0.60)	Age, age at menopause, BMI, physical activity, smoking status	Peplonska et al. (2016)
Estrogen	Japan Hospital nurses Repeated measures study design	Rapid backward RS including DS, ES, and NS; 3 pregnant and 6 non-pregnant nurses	– (for time of the day and type of shift)	None	Yamauchi (2004)

Table 4.11 (continued)

End-point	Location, setting, study design	Exposure level, and number of exposed cases	Response (significance)	Covariates controlled	Reference
Estrogen, LH, FSH	USA Nurses, F Cross-sectional	323 nurses (172 NS and 151 DS) with regular menstrual cycles (no hormone use in 30 d before screening)	Results within NS nurses: Day sleep > night sleep for estrogen ($P < 0.01$), LH ($P < 0.001$), and FSH ($P < 0.001$) Night work > night sleep for estrogen ($P < 0.05$), LH ($P < 0.05$), and FSH ($P < 0.05$) Results comparing NS to DS nurses: NS (during day sleep) > DS (during night sleep) for LH and FSH levels ($P < 0.01$); no difference in estrogen level – (night sleep estrogen, LH, or FSH levels) NS (working) > DS (sleeping) for night LH and night FSH ($P < 0.01$); no change in night estrogen levels	Age, hours of darkness, BMI, number of pregnancies, number of alcoholic beverages consumed, use of psychotherapeutics	Davis et al. (2012)
Prolactin, LH, FSH	Japan Multiple workplaces 1-d trial	Rest day vs night work shift; 5 NS nurses and 6 resting nurses	Decreased prolactin level in NS workers at 02:00 ($P < 0.05$) but not at 22:00; no difference in LH and FSH	None specified	Miyauchi et al. (1992)
Prolactin	France Healthy volunteers 24-h observation	8 permanent NS workers (observed in laboratory for 24-h period after a NS under usual day sleep conditions), 10 day-active participants (observed in laboratory for 24-h period with an 8-h shift in their usual sleep time)	–	None	Spiegel et al. (1996)
Prolactin	Poland Hospital nurses and midwives Cross-sectional	327 women working in irregularly rotating NS, 330 working in DS only (no hormone replacement therapy)	– (for current shift work status, duration of NS, or NS characteristics)	Time of blood sampling, recent cigarette smoking, season of blood sampling, parity with breastfeeding, age, age at menopause	Bukowska et al. (2015)

Table 4.11 (continued)

End-point	Location, setting, study design	Exposure level, and number of exposed cases	Response (significance)	Covariates controlled	Reference
Prolactin, growth hormone, cortisol, urinary catecholamines	Italy Hospital intensive-care unit 4-d trial	10 F nurses in rapid forward RS observed on 4 consecutive days of work (MS, AS, NS, NS)	Prolactin and growth hormone rapidly responded to the RS; cortisol at 07:00, end of the NS < beginning of the MS ($P < 0.05$); catecholamines, first half of NS > second half of NS ($P < 0.05$)	None	Costa et al. (1997)
Prolactin, cortisol, testosterone	France Oil refinery workers 8-h monitoring (midnight to 08:00)	4 male oil-refinery workers in a fast rotating NS schedule, 6 healthy men synchronized to daytime schedule	NS < DS in overall and night "trough" testosterone levels ($P < 0.001$, $P < 0.05$) and overall prolactin ($P < 0.001$); NS > DS in night "trough" cortisol ($P < 0.01$)	None	Touitou et al. (1990)
Cortisol, testosterone, LH	USA Resident physicians, M Repeated measures collected on 4 d (during initial 2 wk of residency, immediately after vacation, after a 36-h shift including on-call NS in obstetrics, and during a DS in gynaecology)	6 resident physicians (M) after a 36-h shift including night	Vacation > initiation of residency ($P = 0.008$) or after NS in obstetrics ($P = 0.0005$) for testosterone; vacation > after NS in obstetrics for LH ($P = 0.039$); no differences in cortisol except that morning < afternoon after NS in obstetrics; no significant differences for gynaecology	None	Chatterton & Dooley (1999)
Growth hormone	France Volunteers from general population 24-h observation	11 M volunteer permanent NS workers (observed in laboratory for 24-h period after a NS under usual day sleep conditions), 10 male day-active participants (observed in laboratory for 24-h period under usual night sleep conditions) performing polysomnography	–	None	Brandenberger & Weibel (2004)
Cortisol	Ontario, Canada Hospital employees, F Cross-sectional	160 DS, 168 RS	RS on NS < DS (for total diurnal cortisol production, $P < 0.01$) – (for RS on DS vs DS, $P = 0.14$)	Age, education, chronotype	Hung et al. (2016)

Table 4.11 (continued)

End-point	Location, setting, study design	Exposure level, and number of exposed cases	Response (significance)	Covariates controlled	Reference
Cortisol	Sweden Pulp and paper factory Cross-sectional	42 backward rotating NS workers, divided into two groups of satisfaction in the shift schedule; serum level (collected during 07:00–09:00 during a morning shift in the first work period of a cycle and during a morning shift in the seventh work period of the cycle)	– (morning cortisol by shift satisfaction) Decreased morning cortisol level at end of shift cycle in evening chronotypes ($P < 0.01$)	Work period and sleep sufficiency before morning shift	Axelsson et al. (2003)
Cortisol	Denmark Hospital Repeated measures	29 surgeons over 4 consecutive days (pre-call; on-call, 15:30–08:30; post-call day 1; post-call day 2); level in saliva	On-call < pre-call ($P < 0.001$)	None	Amirian et al. (2015)
Cortisol	Finland Media workers Cross-sectional	66 irregular SW, 66 regular DS	In irregular SW, decrease in cortisol/melatonin ratio 8 h after awakening for those with vs without prolonged daytime sleepiness ($P = 0.035$)	None	Lindholm et al. (2012)
Cortisol	Italy Hospital Cross-sectional	23 F nurses in a clockwise rapidly RS schedule, 25 DS nurses; (5 saliva samples collected during a MS after a day off in both groups)	RS < DS for morning (06:00 and 08:00) collection times ($P < 0.05$), but not for subsequent collection times (15:00, 20:00, and 04:00 next day)	None	Bracci et al. (2016)
Cortisol	USA Civil aviation employees Experiment	28 workers (12 M, 16 F) randomly assigned to 2 wk of RS: clockwise (2 EMS, 2 AS, 1 midnight) or counter-clockwise (2 AS, 2 EMS, 1 midnight)	– (cortisol rhythm)	None (randomization)	Boquet et al. (2004)
Cortisol	Italy Hospital intensive-care unit Repeated measurements over 4 consecutive shifts	15 F nurses in rapid forward RS; plasma level measured at the beginning, middle, and end of 4 consecutive shifts (MS, AS, NS, NS)	– (cortisol rhythm); cortisol at 07:00, end of NS < beginning of MS ($P < 0.05$)	None	Costa et al. (1994)

Table 4.11 (continued)

End-point	Location, setting, study design	Exposure level, and number of exposed cases	Response (significance)	Covariates controlled	Reference
Cortisol, testosterone	Denmark Male police officers Crossover intervention study	73 participants ("2+2": 2 consecutive NS followed by 2 consecutive recovery days; "4+4": 4 consecutive NS followed by 4 consecutive recovery days; "7+7": 7 consecutive NS followed by 7 consecutive recovery days)	Delay in phase of the cortisol rhythm by 33 min/d – (testosterone)	None	Jensen et al., 2016
Thyroid hormones	Republic of Korea University hospital employees Repeated measures over 5 yr (2011–2015)	546 F NS (≥ 4 NS/mo), 421 F DS; serum level	NS > DS, TSH level ($P = 0.006$) and risk of subclinical hypothyroidism ($P = 0.022$)	Age, department	Moon et al. (2016)
Thyroid hormones	Italy Hospital employees Cross-sectional	220 rotating NS, 422 DS; M and F	Rotating NS > DS, abnormal anti-TPO antibodies ($P = 0.05$)	Age, sex, smoking, drinking, family history of autoimmune thyroid disease, radiation exposure	Magrini et al. (2006)

–, not significant; aMT6s, 6-sulfatoxymelatonin; AS, afternoon shift; BMI, body mass index; d, day; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; DS, day shift; F, female; FSH, follicle-stimulating hormone; h, hour; LH, luteinizing hormone; M, male; min, minute; mo, month; MS, morning shift; NHS-I, Nurses' Health Study cohort; NHS-II, Nurses' Health Study II cohort; NS, night shift; OC, oral contraceptive; RS, rotating shift; SW, shift work; TPO, thyroid peroxidase; TSH, thyroid-stimulating hormone; vs, versus; wk, week; yr, year.

and androgens, but not estrogens, were higher in night shift workers than in daytime workers after adjusting for potential confounders including menstrual phase; the peak level of androgens was also delayed (Papantoniou et al., 2015). In a study of 263 women working irregular rotating night shifts and 269 women working during days, after adjusting for multiple confounders postmenopausal women engaged in night shift work for more than 15 years had higher estradiol levels than those who had worked night shifts for less than 5 years. Current night shift work status, frequency of night duties, and duration of working night shifts were also positively associated with serum estradiol levels in postmenopausal women with a morning chronotype (Peplonska et al., 2016). In a small trial, including three pregnant and six non-pregnant volunteering nurses engaged in a rapidly backward rotating three-shift schedule (morning, afternoon, night, day off) in Japan, estriol level did not vary with time of urine collection (i.e. during the day shift or the night shift) in within-worker comparisons, or with pregnancy status (Yamauchi et al., 2001; Yamauchi, 2004). In an evaluation of early to mid-luteal phase work and levels of sleep estrone conjugate in urine collected throughout work and sleep periods in 323 premenopausal female nurses (172 night shift and 151 day shift), significantly elevated levels in night shift nurses in daytime sleep (vs in night time sleep) and in night time work among night shift nurses (vs in night time sleep) were observed; however, there were no significant differences in work or sleep estrone conjugate levels between night shift nurses and day shift nurses (Davis et al., 2012) (see also Section 4.1.6(a)(ii) below).

(ii) Prolactin, luteinizing hormone, follicle-stimulating hormone, and androgens

In a study of five female nurses during their night shift and six female nurses on their resting day in Japan, nurses working night shifts had significantly lower plasma concentrations of prolactin (PRL) at 02:00 (but not at 22:00), although plasma concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) did not differ between the two groups (Miyauchi et al., 1992). There was also a significantly higher incidence of irregular menstrual cycle in women working at night in a comparison of surveyed teachers, office workers, nurses, factory workers, and barmaids in the same report. [The Working Group noted that the study size was too small for any reliable inference to be drawn.] In a trial of volunteering workers, 8 engaged in permanent night work and 10 in day work, no differences in the plasma PRL profile were observed over two 24-hour sleep–wake cycles (Spiegel et al., 1996). In a study of 327 hospital nurses and midwives working irregularly rotating night shift schedules compared with 330 nurses and midwives working day shifts only, there was no significant association between type of night shift or sleep characteristics and serum PRL level (Bukowska et al., 2015). Plasma PRL immediately responded to alterations to sleep-activity cycle in 10 young female nurses of an intensive care unit engaged in an 8-day rapid forward rotating shift schedule (1 afternoon, 2 mornings, 1 afternoon, 2 nights, 2 rest days) including 1 or 2 nights, but did not differ between measurements taken at the same time on different days (Costa et al., 1997). On the contrary, a significant decrease in PRL level was observed in four male oil refinery workers monitored every 2 hours during their night shift (Touitou et al., 1990). [The Working Group noted that the role of pituitary hormones, such as PRL and growth hormone (discussed below), in cancer of the breast has been investigated less extensively. PRL is released by the anterior lobe of the pituitary gland, but also by the mammary gland, lymphocytes, uterus, prostate, and placenta. By interacting with specific receptors, PRL induces a protein (prolactin-induced protein or PIP) that stimulates DNA synthesis, epithelial cell proliferation, and milk production in the breast (Froes Brandao et al., 2016). In turn,

PIP promotes the growth of breast cancer cells and has a role in facilitating metastasis (Naderi, 2015; Shemanko, 2016).]

Compared with measurements during a period on vacation, plasma LH was decreased in six male resident physicians after a 36-hour work shift, including the night, in an obstetrics ward. No significant changes were observed when the same physicians worked at a gynaecology ward (Chatterton & Dooley, 1999).

Testosterone levels were also monitored in the above study of six male resident physicians. In these men, plasma testosterone levels were suppressed after a night on call at the obstetrics ward and during the first 2 weeks of residency compared with during a vacation period. No significant variations were observed while on-call at the gynaecology ward (Chatterton & Dooley, 1999). In the study of 172 premenopausal night shift nurses and 151 premenopausal day shift nurses (see also Section 4.1.6(a)(i) above), there were significantly elevated urinary LH and FSH in night shift workers in daytime sleep (vs night sleep) and in night work (vs night sleep), when comparing levels during day sleep (for night shift workers) with those in night sleep (for day shift workers), and when comparing levels in night work (for night shift workers) with those in night sleep (for day shift workers) (Davis et al., 2012). In the study of four male oil refinery workers who were engaged in fast rotating shift work, testosterone rhythm was erratic and, at the times of peaks and trough, the serum levels were significantly lower in shift workers than in controls (Touitou et al., 1990). No effect on serum testosterone was observed in the Japanese study of 177 postmenopausal women who ever worked night shifts (Nagata et al., 2008); however, serum testosterone was higher in both men and women working rapid and slow rotating night shifts in the Spanish study covering different trades (Papantoniou et al., 2015), and it was reduced independently of menopausal status, significantly so in the luteal phase, in the study by Gómez-Acebo et al. (2015). [The Working Group noted that the role of testosterone in carcinogenesis is controversial (Klap et al., 2015). Further, whether FSH and LH play a role in modulating the risk of cancer is unclear (Nagamani et al., 1992).]

(iii) Growth hormone

Growth hormone (GH) has been indicated as a contributor to the development, progression, and metastasis of cancer of the breast (Subramani et al., 2017). One study explored the relationship between night shift work that resulted in disruption of the circadian rhythm and changes in the daily rhythm of GH release. GH was monitored every 10 minutes while performing polysomnography in supine posture and receiving enteral nutrition in 11 male workers with at least 2 years of permanent engagement in night shift work at a frequency of 4–5 times per week, and in 10 day workers. The total amount of GH secreted over 24 hours, as well as the mean plasma GH levels, did not vary between the day-active and the night-active workers. In addition, in permanent night shift workers the sleep-related decrease in GH release was compensated for by its increase at varying, unpredictable moments during the waking hours, without any correlation with plasma melatonin levels (Brandenberger & Weibel, 2004). In 10 young female nurses of an intensive care unit, plasma GH rapidly responded to changes in sleep–activity cycle, although its peak at night was less pronounced (Costa et al., 1997).

(iv) Cortisol

Plasma cortisol was suppressed after a night on call, and became normal or elevated in the afternoon in resident doctors in the USA (Chatterton & Dooley, 1999). Cortisol rhythm also had a lesser amplitude, but serum levels at midnight were more elevated in oil refinery night shift workers than in daytime workers (Touitou et al., 1990). Similarly, in a study of female hospital employees

(160 day workers and 168 rotating shift workers) from Ontario, Canada, diurnal cortisol curves were flatter in shift workers on the night shift. In addition, cortisol was decreased in shift workers on the night shift, but cortisol production in shift workers on their day shift was similar to that of day workers (Hung et al., 2016). In a study of 42 shift workers at a paper and pulp factory engaged in rapid backward rotating shift work schedule (1 night, 1 afternoon, and 1 morning, repeated 7 times; then 1 week of rest), participants with an evening chronotype had lower morning cortisol levels at the end of a shift cycle than participants with a morning chronotype (Axelsson et al., 2003). Thirty surgeons who rotated rapidly over two shifts (08:30–15:30, 15:30–08:00, day off) were monitored for 4 days starting at 07:00 on the day shift. In the 29 participants from whom data were collected, cortisol levels manifested a regular rhythm with reduced levels at night and increased levels in the morning hours, although reduced compared with pre-call values (Amirian et al., 2015). Some type of shift rotation was also relevant in influencing adaptation of and levels of cortisol and other hormones. For instance, irregular work shifts in 70 media workers resulted in insufficient recovery and an associated decrease in the salivary cortisol:melatonin ratio (Lindholm et al., 2012). In addition, salivary cortisol levels in the morning hours were lower in 23 nurses employed in clockwise rapidly rotating shift work schedule (1 day, 1 afternoon, 1 night, 2 rest days) compared with 25 nurses working day shifts (Bracci et al., 2016). However, cortisol rhythm was unaffected by rotating shifts, whether clockwise or counter-clockwise, in 28 men and women evenly distributed at random between the two groups (Boquet et al., 2004), or by regular rapidly rotating shifts (2 afternoon, 2 morning, 1 afternoon, 2 nights, 2 rest days) in 15 young female nurses working in an intensive care unit (Costa et al., 1994). In a study of 73 police officers, an increasing number of night shifts altered the diurnal rhythm of cortisol but not testosterone (Jensen et al., 2016). [The Working Group noted that cortisol activates DNA-damaging free radical production, and impairs apoptosis and DNA repair processes, which prevent the progression of abnormal cells towards cancer development (Spiers et al., 2015).]

(v) Thyroid hormones

In a study in the Republic of Korea (n = 967), female night shift workers (4 night shifts/month at a university hospital) demonstrated significantly (P = 0.006) higher levels of thyroid-stimulating hormone (TSH) than those of non-night shift workers, and a 1.4-fold excess risk (P = 0.022) of subclinical hypothyroidism after adjusting for age and hospital department (Moon et al., 2016). The hypothesis of an autoimmune response predisposing to hypothyroidism was investigated by examining anti-peroxidase thyroid antibodies in 220 shift workers engaged in a rotating shift schedule including nights, and 422 daytime workers. After taking into account age, sex, smoking habits, alcohol intake, familial history of autoimmune thyroid disease, and exposure to radiation as possible confounders, shift workers had an increase of more than 2-fold in subclinical autoimmune hypothyroidism (Magrini et al., 2006). [The Working Group noted that in addition to regulating metabolism, development, and growth, thyroid hormones can also stimulate cancer cell proliferation (Lin et al., 2016).]

(b) Experimental systems

Data from studies that evaluated changes in endocrine function in response to alterations in the light–dark schedule in experimental systems are compiled in Table 4.12.

Several studies evaluated serum hormone levels in nonhuman primates exposed to alterations in the light-dark schedule. Capuchin monkeys exposed to continuous light for approximately the last 50 days of gestation had decreased maternal plasma melatonin concentration, and no significant change in either

maternal plasma estradiol or cortisol concentration (Torres-Farfan et al., 2004; Torres-Farfan et al., 2006; Richter et al., 2018). In contrast, increased cortisol concentration in the offspring was observed when the dam was exposed to continuous light during part of the pregnancy. Rhesus macaques maintained on a schedule of 12 hours of light and 12 hours of darkness (LD12:12) had elevated plasma melatonin and progesterone concentrations at night, whereas estradiol, estrone, and cortisol reached peak concentrations in the early morning. Lights were then left on for 12 days, resulting in decreased plasma melatonin concentration but no effect on other steroids (Matsumoto et al., 1991). [The Working Group noted that these studies using capuchin monkeys were of small sample sizes, and that some studies duplicated previously reported data.]

Female Sprague-Dawley rats with mammary adenocarcinomas induced by 7,12-dimethylbenz[*a*]anthracene (DMBA) were exposed to either LD12:12, continuous light, or partial or dim light during the period of darkness (Cos et al., 2006). Rats exposed to light during the period of darkness, especially those under dim light during the period of darkness, had higher tumour growth rates, increased serum estradiol concentration, and lower nocturnal excretion of aMT6s (Cos et al., 2006). Mendez et al. (2012) examined rat maternal and fetal endocrine status as a result of exposure to continuous light during the second half of gestation. Exposure of pregnant dams to continuous light during the second half of gestation had no effect on maternal corticosterone production, despite suppressing maternal melatonin rhythm (Mendez et al., 2012). This maternal exposure to continuous light also retarded intrauterine growth and affected fetal adrenal function in several ways, including changing adrenal clock gene expression and corticosteroid rhythm. Altered corticosteroid rhythm was also observed in mice undergoing shifts in the light–dark schedule (Filipski et al., 2004). Fantie et al. (1984) reported that male Long-Evans rats exposed to either continuous light or continuous dim red light for 60–90 days had higher serum prolactin concentrations or higher serum androgen levels, respectively, compared with controls maintained at LD12:12.

Alterations in the light–dark schedule can also alter other receptor-mediated processes (Table 4.13). For instance, Kettner et al. (2016) demonstrated that advances in the light–dark schedule deregulate nuclear receptor-controlled cholesterol/bile acid and xenobiotic metabolism. Transgenic mice lacking the farnesoid X receptor have increased hepatic bile acid concentrations and, when exposed to shifts in the light–dark schedule, an increased incidence of hepatocellular carcinoma. A reduction in hepatocarcinogenesis was seen in transgenic mice with reduced expression of the constitutive androstane receptor (Kettner et al., 2016). Data relevant to key characteristics of carcinogens from studies that evaluated other receptor-mediated effects after alterations in the light–dark schedule (e.g. Szántóová et al., 2011; Van Dycke et al., 2015; Kochan et al., 2016; Marti et al., 2017) are compiled in Table 4.13.

Aryl hydrocarbon receptor activation affected the expression of clock genes and their response to alterations in the light–dark schedule (Jaeger & Tischkau, 2016). Disruption of *Per1* gene expression in the mammary gland, liver, and haematopoietic cells increased 2,3,7,8-tetrachlorodibenzo-*para*-dioxin-induced CYP1A1 and CYP1B1 expression (Garrett & Gasiewicz, 2006; Qu et al., 2007, 2009).

4.1.7 Alters cell proliferation, cell death, or nutrient supply

(a) Humans

No data were available to the Working Group.

Table 4.12 Effects on endocrine function in response to alterations in the light–dark schedule in experimental systems

Experimental system	Exposure	Relevant finding(s)	Reference
Pregnant capuchin monkey (*Cebus apella*)	Control: LD14:10 Treatment: continuous light from gestation day 100–150 (approximately)	↓ Maternal plasma melatonin No effect on maternal plasma estradiol or cortisol concentration ↑ Plasma cortisol concentration in the offspring	Torres-Farfan et al. (2004)
Pregnant capuchin monkey (*Cebus capucinus*)	Control: LD14:10 Treatment: continuous light from gestation day 100–140 (approximately)	↓ Maternal plasma melatonin ↑ Fetal adrenal 3β-HSD mRNA and progesterone No effect on fetal plasma cortisol or cortisone concentration	Torres-Farfan et al. (2006)
Pregnant capuchin monkey (*Cebus capucinus*)	Control: LD14:10 Treatment: continuous light from gestation day 100 to delivery (approximately 57 d)	↑ Maternal plasma melatonin No effect on maternal plasma cortisol concentration ↑ Newborn plasma cortisol and ↓ DHEAS concentrations; ↓ StAR, 3β-HSD mRNA, and protein expression in adrenal explants of offspring at age 10 mo	Richter et al. (2018)
Rhesus monkey (*Macaca mulatta*) (F)	Control: LD12:12 Treatment: continuous light for 12 d	↓ Plasma melatonin No effect on progesterone, estradiol, estrone, or cortisol concentrations	Matsumoto et al. (1991)
Sprague-Dawley rat (F)	Control: LD12:12 Treatment: continuous light, with dim light during the entire 12-h period of darkness	Light during period of darkness: ↓ nocturnal excretion of aMT6s; changes in the light–dark schedule altered estrous cyclicity Continuous light: ↑ serum estradiol levels ↑ Tumour growth in all groups versus control	Cos et al. (2006)
Sprague-Dawley rat (F)	Control: LD12:12 Treatment: continuous light during second half of gestation	Maternal responses: ↓ maternal plasma melatonin; no effect on maternal plasma corticosterone concentration Fetal responses: ↓ fetal weight, adrenal gland corticosterone, corticosterone response to ACTH, and adrenal gland expression of both MT_1 and *Egr1*	Mendez et al. (2012)
Adult Long-Evans rat (M)	Control: LD12:12 Treatment: continuous white light or continuous darkness (red dim light) for 60–90 d	Both groups: ↓ intromission and ejaculation Continuous darkness: ↑ serum androgen levels Continuous light: ↑ serum prolactin levels	Fantie et al. (1984)
B6D2F$_1$ mouse inoculated with Glasgow osteosarcoma	Control: LD12:12 Treatment: advance in the light–dark schedule by 8 h for 10 d	Disrupted, biphasic corticosterone profile ↓ Hepatic and tumour expression of both *Per2* and *Rev-erb* Enhanced tumour growth	Filipski et al. (2004) (see also Section 4.2.2)

↑, increase; ↓, decrease; 3β-HSD, 3β-hydroxysteroid dehydrogenase; ACTH, adrenocorticotropic hormone; aMT6s, 6-sulfatoxymelatonin; d, day; DHEAS, dehydroepiandrosterone sulfate; *Egr1*, early growth response protein-1; F, female; h, hour; LD, light–dark schedule, light(h):darkness(h); M, male; mo, month; mRNA, messenger RNA; MT_1, melatonin receptor-1; *Per2*, period 2 clock gene; *Rev-erb,* circadian nuclear receptor gene; StAR, steroidogenic acute regulatory protein.

Table 4.13 Other effects on receptor function seen in response to alterations in the light–dark schedule in experimental systems, mapped to selected key characteristics of carcinogens

Key characteristic	Experimental system	Exposure	Relevant finding(s)	Reference
Alters cell proliferation, cell death, or nutrient supply Modulates receptor-mediated effects	C57BL/6J inbred wildtype, *Per1–/–;Per2–/–*, *Cry1–/–;Cry2–/–*, *Albcre;Bmal fl/fl*, *Car–/–*, and *Fxr–/–* mice	Control: LD12:12 Treatment: advances in the light–dark schedule by 8 h (up to 90 wk)	↓ Lifespan with ↑ incidence and earlier onset of NAFLD ↑ Both numbers and sizes of tumours in *Per* and *Cry* mutants, and also the size of tumour in HCC-bearing *Albcre;Bmal fl/fl* mice Deregulation of nuclear receptor-controlled cholesterol, bile acid, and xenobiotic metabolism in the livers of exposed wildtype mice at all ages studied; suppression of FXR and induction of CAR in HCCs; upregulation of transcription factors stimulating cell proliferation and steatosis including β-catenin, c-Myc, Srebp1, Pparγ, Cyp2B10, and Cyp7A1	Kettner et al. (2016)
Alters DNA repair or causes genomic instability	Sprague-Dawley rat (F)	Control: LD12:12 Treatments: advance in the light–dark schedule by 3 h each day for 6 d; advance in the light–dark schedule by 3 h each day for 6 days followed by a 10-d regular LD12:12 for a total of 54 d (6–10–6–10–6–10–6)	After 2 wk (tissues collected 19 h after lights on): base-excision repair, homologous recombination, mismatch repair, and nucleotide-excision repair suggest ↓ DNA repair; ↓ Tp53 signalling and ↓ apoptosis	Kochan et al. (2016)
Modulates receptor-mediated effects	Wistar and Sprague-Dawley rat (M)	Control: LD12:12 Treatment: forced activity for 8 h/d during either normal active or rest periods, for 3 d	Forced activity during normal rest period: ↓ phosphorylation of cap-bound Bmal1 and S6K1 reduced in the PFC; ↓ PFC synaptic ARC protein	Marti et al. (2017)
Modulates receptor-mediated effects	Wistar rats (M)	Control: LD12:12 Treatment: shift (delay) in the light–dark schedule by 8 h every 2 d, for 10 wk	Change in clock gene expression and phase shift in metabolic genes (e.g. *Rev-erbα*, *Pparα*, and *Pdk4*) in the liver and heart	Szántóová et al. (2011)
Modulates receptor-mediated effects	FVB mouse (F)	Control: LD12:12 Treatment: shift (advance or delay) in the light–dark schedule by 8 h every 5 d; samples collected after 1 shift and 5 d recovery, or 6 shifts and 14 d recovery	Corticosterone serum levels affected by both treatments Multiple genes were differentially expressed in the liver from mice from both treatment groups, including: *Cyp2c29*, *Cyp2b10*, *Ntrk2* (kinase signalling), *Tusc3*, *Armcx3* (tumour suppression), and *Gspt2* (cell-cycle progression), among others	Van Dycke et al. (2015)

↑, increase; ↓, decrease; ARC, activity-regulated cytoskeleton-associated protein; Armcx3, armadillo repeat-containing X-linked 3; Bmal1, brain-and-muscle aryl hydrocarbon nuclear translocator (arnt)-like protein-1; CAR, constitutive androstane receptor; Cyp, cytochrome P450; d, day; F, female; FXR, farnesoid X receptor; Gspt2, G1 to S phase transition 2; h, hour; HCC, hepatocellular carcinoma; h, hour; LD, light–dark schedule, light(h):darkness(h); M, male; NAFLD, non-alcoholic fatty liver disease; Ntrk, neurotrophic tyrosine receptor kinase; pdk4, pyruvate dehydrogenase kinase 4; PFC, prefrontal cortex; Ppar, peroxisome proliferator-activated receptor; *Rev-erb*, circadian nuclear receptor; S6K1, S6 kinase β-1; SREBP1, sterol regulatory element-binding protein-1; Tusc3, tumour suppressor candidate-3; wk, week.

(b) Experimental systems

Several studies directly evaluated tumour growth after alteration in the light–dark schedule; these studies are discussed below and in Section 4.2. Additional information can also be found in Section 3 of the present monograph.

Altering the light–dark schedule in mice can result in hepatic pathology, elevated liver enzymes, and increased hepatocyte and bile duct proliferation as assessed using histochemical markers of cell proliferation (e.g. cytokeratin 19, Ki67) (Kettner et al., 2016) (see Section 3.1 and Table 4.13). Blask et al. (2014) showed that DNA synthesis was increased in MCF-7 breast cancer xenografts implanted in rats exposed to light during the period of darkness (see Section 3.5). Tumour growth and cell proliferation (measured using proliferating cell nuclear antigen, PCNA) was increased in rats exposed to light during the period of darkness (Wu et al., 2011) (see Section 3.5). Tumour PCNA levels remained high throughout the entire 24-hour period in rats exposed to light during the period of darkness. Male outbred rats given five subcutaneous injections of 1,2-dimethylhydrazine and kept under continuous light had increased tumour cell proliferation (assessed by PCNA) compared with rats kept at LD12:12 (Panchenko et al., 2008) (see Section 3.4 for additional details).

A single study evaluated the Warburg effect in rats undergoing alterations in the light–dark schedule (Blask et al., 2014). In this study, female nude rats (Hsd:RH-*Foxn1[rnu]*) were exposed to either LD12:12 (control) or to dim light during the period of darkness and, after 6 weeks of exposure, implanted with MCF-7 human breast cancer xenografts (Blask et al., 2014) (see Section 3.5). Exposure continued until tumours reached approximately 5–6 g. The Warburg effect was assessed by evaluating tumour uptake of arterial blood glucose coupled with the release of lactate into the tumour venous blood. Exposure to light during the period of darkness disrupted the rhythms seen in tumour cyclic adenosine monophosphate levels, total fatty acid uptake, linoleic acid uptake, and 13-hydroxyoctadecadienoic acid. Levels of each of these parameters remained significantly elevated over the 24-hour period. [The Working Group noted that there were concerns related to the evaluation of a xenograft tumour model.] Kettner et al. (2016) evaluated changes in glucose metabolite (e.g. glucose 6-phosphate) concentrations in the serum and liver of mice that had been exposed to prolonged alterations in the light–dark schedule, observing the occurrence of accelerated cytoplasmic glycolysis in response. Altered glucose (and glutamine) metabolism was also reported in K-$ras^{LSL\text{-}G12D/+}$;$p53^{flox/flox}$ mice. *Per2* mutant cells had increased rates of glucose consumption and increased levels of lactate excretion compared with $Per2^{+/+}$ cells (Papagiannakopoulos et al., 2016) (see also Section 4.2.2(b)). [The Working Group noted that the circadian clock is critical to metabolism (reviewed by Eckel-Mahan & Sassone-Corsi, 2013; Dibner & Schibler, 2015; Panda, 2016; Maury, 2019). Changes in glucose metabolism are seen in tumour cells, a reliance on glycolysis known as the Warburg effect (Xu et al., 2015). The Working Group's review focused on changes in tumour cell glucose metabolism in response to alterations in the light–dark schedule of experimental systems.]

Blask et al. (2005) performed several studies in situ where tumours were perfused with blood collected from human volunteers exposed to different intensities of light, resulting in different melatonin levels. Human breast cancer xenografts and rat hepatomas perfused in situ with melatonin-rich blood had decreased cell proliferation and linoleic acid uptake and/or metabolism. Tumours perfused with melatonin-deficient blood had higher tumour cell proliferation (Blask et al., 2005) (see also Section 4.2.1(b)). [The Working Group noted that other factors may also have been present in the blood of the human volunteers.]

Fewer studies have used the measurement of PCNA, DNA synthesis, or [^{3}H]thymidine incorporation or a similar approach to directly measure cell proliferation after changes in clock gene expression (e.g. Yang et al., 2009; Lee et al., 2010) (see also Section 4.2.2(b)) or alterations in the light–dark schedule in experimental systems. Shah et al. (1984) showed that exposure of female Holtzman rats to continuous light (24 hours per day) immediately after birth significantly increased DMBA-induced mammary gland cell proliferation as measured using incorporation of [^{3}H]thymidine.

4.1.8 Causes immortalization: changes in telomere length

(a) Humans

See Table 4.14.

Human telomerase reverse transcriptase (*TERT*) mRNA expression, and therefore telomerase activity, oscillates with circadian rhythm under the control of the CLOCK-BMAL1 heterodimer; on the other hand, CLOCK deficiency causes loss of rhythmic telomerase activity, TERT mRNA oscillation, and shortened telomere length (Chen et al., 2014). In a study of 27 physicians, 14 working in emergency departments with night shifts and 13 working in non-emergency departments without night shifts, leukocytes of emergency physicians had a similar low telomerase activity at 10:00 and 17:00. This result indicates loss of the circadian rhythm of telomerase activity (Chen et al., 2014). [The Working Group noted that the authors did not specify the type of shift roster or the sex of the participating physicians.] In a study of 608 employed women and 240 unemployed or part-time working women in the USA, 15 years or more of rotating shifts or night work was not significantly related to relative leukocyte telomere length shortening (Parks et al., 2011). Telomere length in peripheral blood leukocytes was reduced, but not significantly so, after adjustment for age, BMI, and cigarette smoking in female nurses participating in the Nurses' Health Study (NHS-I) with a longer history of night shift work (Liang et al., 2011). Such an effect was seen in women aged 50 years or younger, but not in those older than 50 years. In a nested case–control study of Norwegian nurses including 563 cases of cancer of the breast and 619 controls, telomere length was reduced (–3.18; 95% CI, –6.46 to –0.58; $P = 0.016$) in those working 6 consecutive nights or more for 5 years or more compared with those who only worked day shifts (Samulin Erdem et al., 2017a). Telomere shortening was associated with an increased risk of cancer of the breast in nurses with a long duration of intense night shift work, in terms of number of consecutive nights. Conversely, nurses with longer telomere lengths working 4–5 consecutive nights for 5 years or more had a lower risk of cancer of the breast (Samulin Erdem et al., 2017a).

(b) Experimental systems

One study in young Sudanian diurnal grass rats (*Arvicanthis ansorgei*) showed that alterations in the light–dark schedule were associated with telomere shortening (Grosbellet et al., 2015); see also Section 4.1.1(b) and Table 4.2.

4.1.9 Studies of multiple key characteristics

(a) Humans

[The Working Group noted that metabolism and circadian rhythms are linked at a transcriptional level, although it is unclear whether such effects are under direct CLOCK control, or are mediated by the rest–activity cycle and the timing of food intake.] In a trial in 10 healthy unmedicated male volunteers conducted under controlled conditions of enforced posture, continuous dim light, hourly isocaloric meals, and sleep deprivation for 40 hours, about 15% of all identified metabolites, notably fatty acids in plasma and amino acids in saliva, were under direct circadian control (Dallmann et al., 2012).

Table 4.14 Effects on telomere length in shift workers

End-point	Location, setting, study design	Exposure level, and number of exposed cases	Response (significance)	Covariates controlled	Reference
Telomerase activity	Taiwan, China Volunteering physicians Cross-sectional	14 physicians from emergency department, 13 physicians from non-emergency departments (sex not specified)	Loss of circadian oscillation of telomerase activity in emergency physicians; mean telomerase activity at 10:00 > at 17:00 ($P < 0.001$) in non-emergency but not in emergency physicians after a NS ($P > 0.05$)	None	Chen et al. (2014)
Telomere length	USA Sister study Cross-sectional	608 women, currently employed or with a moderate and/or substantial past employment history, 94 with ≥ 1 yr of RS and 152 with ≥ 1 yr of NS	– (for duration of RS or NS employment)	Age, race, current smoking status, perceived stress, BMI, sleep, physical activity, health status, cardiovascular disease, diabetes, marital status, education, number of children, child in household, total number of years worked	Parks et al. (2011)
Telomere length	USA Nurses' Health Study (NHS-I) Cross-sectional	2409 female nurses with a history of rotating NS (≥ 1 yr), 1583 female DS nurses	– (for duration of rotating NS, $P = 0.36$)	Age, BMI, cigarette smoking	Liang et al. (2011)
Telomere length	Norway Hospital nurses Nested case–control study	563 breast cancer cases and 619 controls; 96 nurses worked ≥ 6 consecutive nights for ≥ 5 yr and 166 DS nurses	Telomere shortening ($P = 0.016$) with NS	Not reported	Samulin Erdem et al. (2017a)

–, not significant; BMI, body mass index; DS, day shift; NHS-I, Nurses' Health Study cohort; NS, night shift; RS, rotating shift.

Patterns of human plasma proteins were assessed in six healthy male participants during daytime food intake and night sleep while in accordance with the circadian rhythm, versus daytime sleep and night food intake under conditions of simulated night shift work. A total of 127 proteins were altered during simulated night shift work, associated with immune function, metabolism, and cancer. Of these, 30 proteins were observed to be strongly regulated by the circadian cycle; these were associated with pathways involved in extracellular matrix organization, tyrosine kinase signalling, and signalling by receptor-tyrosine protein kinase erbB-2 (Depner et al., 2018).

(b) Experimental systems

Kettner et al. (2016) performed microarrays on total liver RNA from control mice (LD12:12), and mice exposed to shifts in the light–dark schedule, at age 12 and 30 weeks (see Table 4.13 for a partial description of the results; see also Table 3.1). This study also included a circadian metabolomics study of serum and hepatic carnitines, lipids and prostaglandins, coenzyme A, and tricarboxylic acid cycle metabolites in wildtype mice at age 12 and 30 weeks. Kochan et al. (2016) investigated the effect of various alterations in the light–dark schedule on mammary gene expression in Sprague-Dawley rats (see Table 4.13). In this study, control rats were exposed to LD12:12 for 6 or 54 days after an initial acclimation period. In the first treatment group, the period of light was initiated 3 hours earlier each day for 6 days (only). In the second treatment group, the 6 days of 3-hour advances in the light–dark schedule were followed by 10 days of LD12:12, followed by another 6 days of 3-hour advances in the light–dark schedule, for a total of 54 days (6, 10, 6, 10, 6, 10, 6). Their analysis revealed significant changes in gene expression at a single time-point (at 2 weeks and 19 hours after the initiation of the period of light). Gene expression changes observed at this time were associated with disturbances in base-excision repair, homologous recombination, mismatch repair, and nucleotide-excision repair processes. Gene expression patterns correlating with decreased Tp53 signalling, decreased DNA repair, and decreased apoptosis were also observed. [The Working Group noted that these results should be interpreted with caution because changes in gene expression occurred in only a single group, sample sizes were small, and no exposure duration-response relationship was observed.] Livers from female FVB mice exposed to six shifts in the light–dark schedule in both clockwise and counter-clockwise directions were evaluated using microarray approaches (Van Dycke et al., 2015; see also Table 4.13 and Table 3.1). Shifts in the light–dark schedule led to changes in hepatic expression of *Cd36*, *Ntrk2*, *Igh-VJ558*, *Srgap3*, *Tram1*, *Snrpn*, *Rbp1*, *Cyp2b10*, and *Cyp2c29*. Several of these genes (e.g. *Cd36*, *Ntrk2*) were postulated as being associated with cancer of the breast. Wu et al. (2012) investigated the effect of advances in the light–dark schedule on tumour growth and metastasis in male C57BL/6 mice (see also Table 3.2). In this study, Lewis lung carcinoma cells were inoculated into mice exposed for 10 days to either LD12:12 (control) or to advances in the light–dark schedule by 8 hours every 48 hours for 37 days (inoculation on day 10). cDNA microarrays and real-time quantitative reverse transcription polymerase chain reaction on liver and tumour cells were used to assess gene expression. Tumours grew faster in the mice exposed to advances in the light–dark schedule than in control mice. Microarray data showed that, in both liver and tumours, there was altered expression of genes related to the cell cycle, apoptosis, immune response, and metastasis suppressor genes. The expression of the *Ndrg1* gene was suppressed by advances in the light–dark schedule. Continuous gestational light in rats has also been reported to induce modification of the fetal liver transcriptome representing a diverse set of pathways,

including haematopoiesis, coagulation cascade, complement system, and carbohydrate and lipid metabolism (Spichiger et al., 2015).

4.2 Other relevant evidence

4.2.1 Melatonin

(a) Humans

This section focuses on studies with a sample size of more than 10 that assessed melatonin levels and had stronger design features (including the timing and multiplicity of biosample collection, appropriateness of control group, and characterization of the night shift work exposure). [The Working Group also noted that definitions of shift work varied across the studies. Although most studies collected urine samples for melatonin measurement, the timing and times of sample collection also varied across studies.]

The studies included in this section measured melatonin in the urine or saliva to determine whether shift work can reduce melatonin production (Table 4.15). For example, Schernhammer et al. (2004) measured aMT6s, a major metabolite of melatonin, from morning-void urine samples over a 3-year period in 79 premenopausal women who participated in the Nurses' Health Study cohorts to evaluate potential associations between night work and hormone levels. A significant inverse association was observed between an increasing number of nights worked within the 2 weeks preceding morning-void urine collection and aMT6s levels ($r = -0.30$; $P = 0.008$). A later publication from the same group also reported that melatonin concentrations calculated across 24 hours were similar for different shift work categories, with a variation of these concentrations over the course of the day. In a study set within the NHS-II cohort, rotating shift workers on night shifts had lower levels of urinary melatonin during the night compared with day workers, as well as smaller peaks and later peak onset (Razavi et al., 2019). There was also a significant interaction between rotating shift work and chronotype, with better alignment between rotating shift work and chronotype with less disruption of melatonin rhythms.

Burch et al. (2005) also measured and compared melatonin production, light exposure, and physical activity levels in 165 manufacturing workers on three non-rotating shifts (first, 06:00–14:00; second, 14:00–22:00; and third, 22:00–06:00). Concentrations of 6-hydroxymelatonin sulfate (6-OHMS), another major urinary metabolite of melatonin, were measured as adjusted mean sleep–work ratios of 6-OHMS concentration normalized to urinary creatinine levels (6-OHMS/cr) in post-shift and post-sleep urine samples. Levels of 6-OHMS were very similar between the first (ratio, 4.2) and the second (ratio, 4.5) shifts, but lower for the third shift (ratio, 2.3).

Davis et al. (2012) conducted the largest study of female nurses, comprising 172 night shift and 151 day shift nurses, to investigate whether night shift work is associated with decreased levels of melatonin. Their results showed that aMT6s levels were 62% lower during the daytime sleep of night shift workers than during the night sleep of day shift workers.

Mirick et al. (2013) performed the largest study in men, including 185 night shift and 158 day shift health-care workers, to investigate whether night shift work affects levels of melatonin. The study found that night shift workers had significantly lower aMT6s levels during daytime sleep (57% lower) than the day shift workers during night sleep.

Findings from 10 other studies also support the association between night shift work and a significantly decreased melatonin level (Hansen et al., 2006; Grundy et al., 2009; Bracci et al., 2013; Dumont & Paquet, 2014; Papantoniou et al., 2014; Amirian et al., 2015; Gómez-Acebo et al., 2015; Jensen et al., 2016; Leung et al., 2016; Daugaard et al., 2017). Studies that examined

Table 4.15 Effects on levels of melatonin in urine or saliva of shift workers

Melatonin or metabolite	Biosample type	Location, setting, study design	Exposure level, and number of exposed and controls	Response (significance)	Covariates controlled	Reference
aMT6s	Post-sleep urine	USA Nurses' Health Studies (NHS-I and NHS-II) (three sample collections over 3 yr) Cross-sectional analysis	79 premenopausal women, number of NS in the last 2 wk (0, 1–4, > 4)	Melatonin decreased with increasing number of NS ($P = 0.008$)	None	Schernhammer et al. (2004)
aMT6s	72-h urinary collection after ≥ 3 d without night shift work	USA Nurses' Health Study II (NHS-II) Cross-sectional analysis	130 female nurses (84 RS and 46 DS)	RS on NS < DS; interaction between RS and chronotype ($P < 0.05$)	Age, BMI, melatonin batch, region (time zone), month of melatonin collection	Razavi et al. (2019)
6-OHMS	Post-sleep urine (creatinine-adjusted)	USA Medical device-manufacturing facility Cross-sectional study	165 workers on non-rotating shifts (71 first shift, 06:00–14:00; 62 second shift, 14:00–22:00; and 32 third shift, 22:00–06:00)	Third < first or second shift ($P < 0.01$)	Month, second-hand smoke, EMF concerns, eye colour, BMI	Burch et al. (2005)
aMT6s	24-h urinary collection	USA Hospital nurses Cross-sectional study	323 female nurses (172 NS and 151 DS)	NS < DS for: day sleep vs night sleep ($P < 0.0001$), night sleep vs night sleep ($P < 0.0001$), and night work vs night sleep ($P < 0.0001$) For NS, day < night sleep ($P < 0.001$) and night work < night sleep ($P < 0.0001$)	Age, hours of darkness, BMI, number of pregnancies, number of alcoholic beverages consumed, use of psychotherapeutics	Davis et al. (2012)
aMT6s	24-h urinary collection	USA Health-care providers Cross-sectional study	343 male health-care workers (185 NS and 158 DS)	NS < DS ($P < 0.0001$)	Age, hours of darkness, BMI, number of alcoholic beverages consumed, nicotine/tobacco use, medication use	Mirick et al. (2013)
aMT6s	24-h urinary collection on a work day and on a day off	Denmark Female nurses Cross-sectional study	89 fixed shift nurses (27 DS, 07:00–15:00; 12 ES, 15:00–23:00; 50 NS, 23:00–07:00)	NS < days off ($P < 0.001$); NS < DS on work days ($P < 0.01$)	Age, BMI, smoking, number of children, age at birth of first child, menopausal status	Hansen et al. (2006)

Table 4.15 (continued)

Melatonin or metabolite	Biosample type	Location, setting, study design	Exposure level, and number of exposed and controls	Response (significance)	Covariates controlled	Reference
aMT6s	One post-sleep urine and four saliva samples over a 24-h period	Canada Female nurses Cross-sectional study	61 RS nurses (29 sampled on DS, 07:00–19:00; 32 sampled on NS, 19:00–07:00)	NS < DS (urinary melatonin, $P = 0.0003$) – (salivary melatonin)	None	Grundy et al. (2009)
aMT6s	Urine collected during 23:00–07:00	Italy Hospital nurses Cross-sectional study	31 healthy premenopausal (not using drug treatments) rapid forward rotating NS in the last 2 mo or longer; 31 DS	NS < DS ($P < 0.05$)	Age, BMI, physical exercise, smoking	Bracci et al. (2013)
aMT6s	24-h urinary collection	Spain Workers from two public hospitals, a car manufacturer, and a railway company Cross-sectional study	113 workers (72 NS and 41 DS)	NS < DS ($P < 0.001$), particularly in NS workers with a morning chronotype	Age, chronotype, education level, sex, menopausal status, parity, age at first full-term birth	Papantoniou et al. (2014)
aMT6s	24-h urinary collection	Canada Simulated NS work in healthy young participants Laboratory study	38 participants (simulated DS, 09:00–17:00; followed by 3 consecutive days of simulated NS, midnight to 08:00)	NS < DS ($P < 0.05$)	Within-person design	Dumont & Paquet (2014)
aMT6s	12-h urinary collection (21:00–09:00) over 4 consecutive days (pre-call; on-call, 15:30–08:30; post-call day 1; post-call day 2)	Denmark Physicians Cross-sectional study	21 surgeons	On-call < pre-call ($P = 0.004$)	None	Amirian et al. (2015)
aMT6s	24-h urinary collection	Spain Health-care workers and teachers Cross-sectional study	136 female workers (73 DS and 63 rotating NS)	NS < DS (significance not reported)	None	Gómez-Acebo et al. (2015)

Table 4.15 (continued)

Melatonin or metabolite	Biosample type	Location, setting, study design	Exposure level, and number of exposed and controls	Response (significance)	Covariates controlled	Reference
aMT6s	Saliva samples collected every 4 h when awake on the last day with NS, and on the last recovery day, for each intervention	Denmark Male police officers Crossover intervention study	73 participants ("2+2": 2 NS followed by 2 recovery days; "4+4": 4 NS followed by 4 recovery days; "7+7": 7 NS followed by 7 recovery days)	Melatonin decreased with increasing number of NS ($P = 0.006$)	None	Jensen et al. (2016)
aMT6s	48-h urinary collection	Canada Female health-care workers Cross-sectional study	261 workers (147 fixed DS and 114 on RS including NS)	NS < DS ($P < 0.05$), particularly in NS workers with a later chronotype	Cumulative shift work, age, education, parity, age at first birth, use of sleep aid	Leung et al. (2016)
aMT6s	Saliva samples collected every 4 h on both a work day and a day off	Denmark Indoor, outdoor, and night workers Cross-sectional study	341 workers (254 DS and 87 NS, 19 of which regular and 68 RS)	NS < DS on work days ($P < 0.05$) but not on days off	Time of day, age, sex, BMI, current smoking, diurnal preference, use of antidepressant medication	Daugaard et al. (2017)
aMT6s	First morning urine void	Japan Breast cancer screening Cross-sectional study	7 Ever vs 170 never worked at night; all postmenopausal women not using hormone replacement therapy	–	Age, BMI, smoking status, alcohol consumption, group of participants, day length before urine collection	Nagata et al. (2008)
aMT6s	Morning urine	USA Nurses' Health Study Cross-sectional analysis	464 women (384 controls from a nested case–control study on urinary melatonin and breast cancer and 80 premenopausal participants from a validation study), of whom 44 had worked ≥ 1 night in the most recent 2 wk and 310 had worked ≥ 1 yr of rotating NS	– (for number of NS worked in the 2 wk before urine collection or years of rotating NS)	Age, BMI, weight change from age 18 yr, alcohol consumption, month of urine collection, antidepressant use, aspirin use, physical exercise, smoking history and pack-years smoked, height, first spot morning urine, sleep duration	Schernhammer et al. (2006)

Table 4.15 (continued)

Melatonin or metabolite	Biosample type	Location, setting, study design	Exposure level, and number of exposed and controls	Response (significance)	Covariates controlled	Reference
aMT6s	24-h urinary collection	UK Oil rig workers Cross-sectional study	34 participants with 2 wk of DS (06:00–18:00) followed by 2 wk of NS (18:00–06:00): 5 drill crew in winter, 6 maintenance crew in winter, 23 maintenance crew in summer	–	None	Barnes et al. (1998a)
aMT6s	24-h urinary collection	Canada Telecommunications workers Cross-sectional study	13 full-time RS (sampled for two 24-hour periods, one including a NS and one including a DS/ES)	–	None	Dumont et al. (2012)
aMT6s	Urine collection at the beginning of a morning shift after a regular night's sleep on a day off	Italy Nurses Cross-sectional study	116 female nurses (60 with ≥ 2 yr of SW and 56 permanent DS)	–	Age, physical activity, number of offspring	Bracci et al. (2014)
aMT6s	24-h urinary collection	UK Oil rig workers Cross-sectional study	18 participants with 1 wk of DS (12:00 to midnight) followed by 1 wk of NS (midnight – 12:00): 11 in November, 7 in March	Mixed results by season	None	Barnes et al. (1998b)

–, not significant; 6-OHMS, 6-hydroxymelatonin sulfate; aMT6s, 6-sulfatoxymelatonin; BMI, body mass index; d, day; DS, day shift; EMF, electromagnetic field; ES, evening shift; h, hour; mo, month; NHS-I, Nurses' Health Study cohort; NHS-II, Nurses' Health Study II cohort; NS, night shift; RS, rotating shift; vs, versus; wk, week; yr, year.

levels of melatonin by chronotype reported inconsistent findings (Papantoniou et al., 2014; Leung et al., 2016).

However, in a study by Nagata et al. (2008), no significant difference was observed in urinary levels of aMT6s between postmenopausal women who had ever and never worked at night. Schernhammer et al. (2006) found no association between short- or long-term rotating night shift work and urinary levels of aMT6s in women in the NHS-II cohort. Several other studies also found no significant difference in total 24-hour aMT6s production between night shift and day shift work periods (Barnes et al., 1998a; Dumont et al., 2012; Bracci et al., 2014); an additional study reported mixed results (Barnes et al.,1998b). [The Working Group noted that the sample sizes of these four studies were relatively small (n = 34, 13, 60, and 18, respectively).]

(b) Experimental systems

Data relevant to key characteristics of carcinogens from studies that evaluated melatonin in experimental systems exposed to alterations in the light–dark schedule are compiled in Table 4.16. Blask et al. (2003) (see Section 3.5.2) demonstrated that rats maintained on LD12:12 exhibited a robust circadian melatonin rhythm that was abolished by exposure to continuous light. Rats inoculated with human MCF-7 breast cancer xenografts and exposed to continuous light had increased tumour growth, increased linoleic acid and its metabolism to the mitogenic molecule 13-hydroxyoctadecadienoic acid (13-HODE), and higher tumour growth rate, suggesting increased cell proliferation. In a series of follow-up studies, Blask et al. (2005) (see Section 3.5.2) showed that exposure of rats bearing rat hepatomas or human breast cancer xenografts to increasing intensities of light during each 12-hour dark phase (0–345 μW/cm^2) resulted in a dose-dependent suppression of nocturnal melatonin blood levels and a stimulation of tumour growth and linoleic acid uptake/metabolism to 13-HODE. Blask et al. (2005) also performed several in situ studies in which tumours were perfused with blood collected from human volunteers exposed to different levels of light. These exposures resulted in different melatonin levels in the blood. In these tumour perfusion studies, human breast cancer xenografts and rat hepatomas perfused in situ with blood high in melatonin (derived from humans exposed to a normal light–dark schedule) had decreased cell proliferation and linoleic acid uptake/metabolism. In contrast, tumours perfused with melatonin-deficient blood (collected from people exposed to light at night) had higher tumour cell proliferation. Nude rats inoculated with MCF-7 human breast cancer xenografts and exposed to dim light during the period of darkness had almost-undetectable blood melatonin levels, more rapid onset of tumour growth, and significantly increased tumour cell proliferation (Dauchy et al., 2014). Female Sprague-Dawley rats given a single oral dose of DMBA at 20 mg and exposed to continuous dim light during the period of darkness had significantly lower melatonin production, as assessed by urinary excretion of aMT6s, and elevated rates of mammary tumour growth (Cos et al., 2006; see Section 4.1.6(b)). A study performed by van den Heiligenberg et al. (1999), which involved the exposure to continuous light of Wistar rats given diethylnitrosamine, reported similar effects on melatonin production and tumour growth rates. In contrast, Travlos et al. (2001) reported that light during the period of darkness also impaired melatonin production in female Fischer 344 rats; however, tumour growth rates (induced by injection of *N*-nitroso-*N*-methylurea) were unaffected by decreased melatonin production.

Dimovski & Robert (2018) reported decreased plasma melatonin in adult tammar wallabies housed under either amber or white light at night (see also Section 4.1.1(b)).

Table 4.16 Melatonin disruption in response to alterations in the light–dark schedule, mapped to selected key characteristics of carcinogens

Key characteristic	Experimental system	Exposure	Relevant finding(s)	Reference
Alters cell proliferation, cell death, or nutrient supply	RNU nude rats (F) implanted with MCF-7 breast cancer xenografts grown in BALB/c nude mice	Control: LD12:12 Treatment: continuous light Melatonin measured after 5 wk; all rats continued on LD12:12 after implantation until day 40, when some tumour-bearing rats were changed to continuous light	↑ Tumour growth ($P < 0.05$), ↑ Tumour LA uptake and its metabolism to 13-HODE (mitogen)	Blask et al. (2003)
Alters cell proliferation, cell death, or nutrient supply	Buffalo (BUF(BUF/Ncr)) rats (M) implanted with rat hepatocarcinoma Nude rats (HSD:RH-rnu) (F) implanted with MCF-7 human breast cancer xenografts	Control: LD12:12 Treatment: variable light intensity during each 12-h period of darkness: 0 (continuous darkness), 0.02, 0.05, 0.06, 0.08, and 345 (continuous light) μW/cm^2; exposure began 2 wk before tumour implantation, and continued until the end of each tumour growth period ± Tumours perfused in vitro with human blood collected from women exposed to various different lighting conditions: daytime; night after 2 h of complete darkness; and night after 90 min of white light exposure	Rat hepatoma expressed both MT_1 and MT_2; tumours expressing MT_1 were responsive to melatonin (e.g. ↓ tumour cAMP levels) Male rats exposed to variable light showed a dose-dependent ↓ in serum melatonin levels Tumour perfusion with blood collected during night: ↓ [^{3}H]thymidine incorporation compared with blood collected during the day; the addition of S20928, a non-selective MT_1/MT_2 antagonist, blocked the tumour-suppressive effects of melatonin-rich blood collected at night	Blask et al. (2005)
Alters cell proliferation, cell death, or nutrient supply	Nude rats (HSD:RH-*Foxn1*^{rnu}) (F) implanted with MCF-7 human breast cancer xenografts; some rats treated with tamoxifen when tumours were ~ 2.5 g	Control: LD12:12 Treatment: dim light during the period of darkness Tumours implanted 1 wk after change in the light–dark schedule; exposure continued until study termination when tumours became larger	↓ Blood melatonin levels ↑ Tumour incorporation of [^{3}H]thymidine Breast tumour xenografts from rats housed in dim light during the period of darkness had ↓ latency-to-onset ($P < 0.001$) and a faster growth rate ($P < 0.001$)	Dauchy et al. (2014)
Alters cell proliferation, cell death, or nutrient supply	Wistar rats (M) Rats given DEN orally at ~ 10 mg/kg bw per d for 6 wk, then either DEN only, DEN + phenobarbital (at 30 mg/d), or continuous light for up to 77 d	Control: LD12:12 Treatment: continuous light	↓ Urinary aMT6s, ↑ hepatic tumour frequency and size ($P < 0.05$)	van den Heiligenberg et al. (1999)
Alters cell proliferation, cell death, or nutrient supply	F344/N rats (F) pineal-intact and pinealectomized Single intraperitoneal injection of NMU at 50 mg/kg bw	Control: LD12:12 Treatment: light during the period of darkness for 1-min intervals at 14:00, 16:00, 18:00, 20:00, and 22:00 Up to 26 wk	↓ Serum melatonin, no effect on the incidence or development of NMU-induced mammary tumours	Travlos et al. (2001)

↓, decrease; ↑, increase; 13-HODE, 13-hydroxyoctadecadienoic acid; aMT6s, 6-sulfatoxymelatonin; bw, body weight; cAMP, cyclic adenosine monophosphate; d, day; DEN, diethylnitrosamine; F, female; h, hour; LA, linoleic acid; LD, light–dark schedule, light(h):darkness(h); M, male; min, minute; MT_1 or MT_2, melatonin receptor 1 or 2; NMU, *N*-nitroso-*N*-methylurea; wk, week.

4.2.2 Disruption of clock genes

(a) Humans

See Table 4.17.

Ten studies in humans, mostly of cross-sectional design, have examined the association between shift work and alterations in the expression of clock genes (i.e. core circadian genes). None of these studies provided data on dysfunction metrics of the molecular circadian clock or any measure of its phase shift or amplitude modification.

Of the 10 studies, 5 measured mRNA expression of clock genes and 5 measured methylation of those genes, which may modulate their expression (Rauch et al., 2009; Bell et al., 2011). The studies varied considerably in terms of eligibility criteria, number of participants, shift schedules evaluated, and control of covariates.

In the studies of clock gene expression, there was a general lack of consistency between studies in terms of the specific clock genes identified and the direction of effects. Limitations of the studies included the small numbers of participants and/or only single time-points being considered (Reszka et al., 2013; Bracci et al., 2014; Fang et al., 2015; Kervezee et al., 2018; Koshy et al., 2019). Expression of clock genes is known to vary over a 24-hour day (Zhang et al., 2014), but multiple measurements throughout a day were not always captured. [The Working Group noted that differences in study design may explain some differences in results.] For example, Bracci et al. (2014) compared clock gene expression in blood between rotating shift workers (≥ 2 years on shift) and day shift workers (see also Section 4.1.6(a)). Since blood samples were collected from the rotating shift workers during a day shift that followed a day off (i.e. not during or immediately after completing a night shift), short-term effects on gene expression were likely to be missed. On the other hand, Kervezee et al. (2018) conducted a laboratory-based study of healthy volunteers that evaluated changes in gene expression over 4 days of simulated night shift work (see also Section 4.1.2(a)). This study highlighted rapid acute disruption of the circadian coordination by simulated night work, but it was unable to address the issue of chronic impacts of long-term shift work on gene expression.

Results of the methylation studies were also mixed in terms of specific clock genes identified and the direction of effects (Zhu et al., 2011; Bhatti et al., 2015; Adams et al., 2017; Samulin Erdem et al., 2017b). Compared with gene expression, DNA methylation is thought to be more stable over shorter periods of time. Thus, capturing multiple measurements over the course of a single day is likely to be unnecessary. [However, DNA methylation is cell specific, so an important consideration of the Working Group was the potential for systematic differences in the composition of tissue samples between comparison groups.] Diurnal variation in blood cell composition, for example, could contribute to observed differences in clock gene methylation. This issue can be addressed through adjustment for cellular composition (Houseman et al., 2012), which was not performed in two of the studies (Zhu et al., 2011; Reszka et al., 2018). In two of the studies, participants may have last performed shift work many years before blood sample collection (Zhu et al., 2011; Samulin Erdem et al., 2017b); [the Working Group noted that it is uncertain how this may have impacted the ability to observe relevant changes in methylation related to shift work].

(b) Experimental systems

Circadian clock genes have been shown to alter cell proliferation and tumour growth. For example, transgenic mice deficient in the circadian clock gene, period homologue 2 (*Per2*), had increased tumour development after gamma radiation (Fu et al., 2002; Fu & Lee, 2003; Lee et al., 2010) and accelerated $Apc^{Min/+}$ tumorigenesis (Wood et al., 2008) compared with wildtype mice. Castanon-Cervantes et al. (2010) (Table

Table 4.17 Clock gene expression and methylation in shift workers

End-point	Biosample type	Location, setting, study design	Exposure level, and number of exposed and controls	Response (significance)	Covariates controlled	Reference
Clock gene expression	Blood	Poland Nurses and midwives Cross-sectional study	92 RS (mean duration NS 24.2 yr), 92 DS (mean duration NS12.0 yr)	RS > DS for *PER1* ($P = 0.03$) NS for ≥ 15 yr > DS for *PER1* ($P = 0.01$)	Hour and season of the year of blood collection	Reszka et al. (2013)
Clock gene expression	Blood	Italy Nurses Cross-sectional study	60 RS (day 1, 07:00–14:00; day 2, 14:00–22:00; day 3, 22:00–07:00) for ≥ 2 yr, 56 DS	RS > DS for *BMAL1* ($P = 0.04$), *CLOCK* ($P = 0.008$), *NPAS2* ($P = 0.012$), *PER1* ($P = 0.008$), *PER2* ($P = 0.047$), *REV-ERBα* ($P = 0.045$) RS < DS for *PER3* ($P = 0.012$), *CRY1* ($P = 0.002$), *CRY2* ($P = 0.005$)	Age, chronotype score, physical activity, number of offspring Multiple eligibility criteria	Bracci et al. (2014)
Clock gene expression	Blood	USA Hospital residents and/or interns Short-term follow-up study	15 participants on 7-d floating NS	NS > DS for *PER2* ($P = 0.03$)	Multiple eligibility criteria; within-person design	Fang et al. (2015)
Clock gene expression	Blood	Canada Healthy men and women in laboratory study	8 participants transitioned from DS to NS	NS < DS for *PER1* amplitude ($P < 0.001$)	Within-person design	Kervezee et al. (2018)
Clock gene expression	Blood and oral mucosa	Location, NR Male and female police officers Short-term follow-up study	11 participants observed over 1 wk of DS and ES and over 1 wk of NS (5 consecutive 9-h nights and 2 12-h nights)	DS or ES (but not NS) demonstrated significantly increased *PER1–2* and *REV-ERBα* acrophase ($P < 0.05$), and significantly decreased *PER1* acrophase ($P < 0.01$) NS vs DS/ES, *PER1* phase shift ($P < 0.0001$)	None	Koshy et al. (2019)
Clock gene methylation	Blood	Denmark Female participants nested in cohort study Cross-sectional study	19 NS (≥ 10 yr), 98 DS (no history of NS)	NS < DS for *CLOCK* ($P = 0.05$); NS > DS for *CRY2* ($P = 0.04$)	Age, total folate intake	Zhu et al. 2011

Table 4.17 (continued)

End-point	Biosample type	Location, setting, study design	Exposure level, and number of exposed and controls	Response (significance)	Covariates controlled	Reference
Clock gene methylation	Blood	USA Female and male health-care workers Cross-sectional study	59 NS (24 h/wk for ≥ 6 mo), 65 DS	NS < DS (FDR ≤ 0.05) for *CLOCK*, *CSNK1D*, *CSNK1E*, *NPAS2*, *NR1D1*, *PER1*, *PER2*, *PER3*, *RORA*	Age, sex, BMI, race, smoking status, leukocyte cell proliferation Multiple eligibility criteria	Bhatti et al. (2015)
Clock gene methylation	Saliva	Norway Female nurses Case–control study	278 breast cancer cases: 70 never NS, 209 ever NS (28 never ≥ 3 consecutive NS; 41 ≥ 3 consecutive NS for < 5 yr; 140 with ≥ 3 consecutive NS for ≥ 5 yr)	Among cases, ≥ 3 consecutive NS < 5 yr > *BMAL1* methylation ($P = 0.003$); ever NS, never ≥ 3 NS and ≥ 3 consecutive NS ≥ 5 yr < *CRY1* methylation ($P = 0.040$); ≥ 3 NS < 5 yr > *PER1* methylation ($P = 0.035$)	Alcohol (BMAL1), familiar breast cancer (CRY1), years since cancer, alcohol consumption (PER1)	Samulin Erdem et al. (2017b)
Clock gene methylation	Blood	USA Female health-care workers Cross-sectional study	111 NS (≥ 20 h/wk, 8 h per shift for ≥ 6 mo), 86 DS	–	Age, BMI, race, alcohol consumption, smoking, leukocyte cell mixture (FDR ≤ 0.05) Multiple eligibility criteria	Adams et al. (2017)
Clock gene methylation	Blood	Poland Nurses and midwives Cross-sectional study	347 RS (including NS 19:00–07:00), 363 DS	RS < DS for *PER2* ($P = 0.004$); more NS per month < fewer NS per month for *PER2* ($P = 0.012$)	Age, current smoking status, folate intake, blood collection time	Reszka et al. (2018)

–, not significant; BMI, body mass index; d, day; DS, day shift; ES, evening shift; FDR, false discovery rate; h, hour; mo, month; NR, not reported; NS, night shift; RS, rotating shift; vs, versus; wk, week; yr, year.

4.18) reported altered immune response to LPS in *Per2*^{Luc} knock-in mice after alteration in the light–dark schedule (see also Section 4.1.3 (b)). *Per2*$^{m/m}$ mice also have increased small intestinal mucosa β-catenin and cyclin D protein levels and increased numbers of colonic polyps compared with wildtype mice (Wood et al., 2008). Mice deficient in *Bmal1* (*Bmal1*$^{+/-}$), *Cry1* and *Cry2* (*Cry1*$^{-/-}$; *Cry2*$^{-/-}$), *Per1* and *Per2* (*Per1*$^{-/-}$; *Per2*$^{m/m}$), or *Per2* alone (*Per2*$^{-/-}$), kept under alternating light–dark conditions (24-hour light-dark cycles) and irradiated had an increased incidence of cancer (Lee et al., 2010) (see also Section 4.1.7(b)).

Relevant studies evaluating clock genes in experimental systems use different environmental light–dark schedules including: (i) 24-hour light–dark conditions; (ii) continuous exposure to either light or darkness; and (iii) other alterations in the light–dark schedule. Collectively, these studies have shown that changes in the light–dark schedule that result in altered clock gene expression have effects on the immune system, cell proliferation including increased tumour growth, and some changes in receptor-mediated function (Table 4.18). Repeated advances in the light–dark schedule in rats can: alter clock gene expression cytokine cyclicity and function (Castanon-Cervantes et al., 2010; Logan et al., 2012); impair natural NK cytolytic activity (Logan et al., 2012); and, in rats inoculated with mammary adenocarcinoma MADB106 tumour cells, have functional consequences expressed as increased tumour growth (Logan et al., 2012). Advances in the light–dark schedule altered neuropeptide, lipid metabolism, inflammation, and endoplasmic reticulum gene profiles in multiple tissues (Herrero et al., 2015). Increased inflammation also occurred in transgenic mice with altered clock gene expression exposed to alterations in their light–dark schedule (Kim et al., 2018). Effects such as decreased expression of Tp53, increased c-Myc expression, and enhanced growth of transplanted tumours in animals undergoing shifts in the light–dark schedules have also been reported (Filipski et al., 2004, see Table 4.12; Filipski et al., 2005, 2006; Papagiannakopoulos et al., 2016).

4.2.3 Vitamin D

See Table 4.19.

Serum 25-hydroxyvitamin D concentrations lower than 50 nmol/L (20 ng/mL) are usually considered to be insufficient for osteogenesis (Coppeta et al., 2018); such a condition has been reported in shift workers and indoor workers (Coppeta et al., 2018). For instance, Itoh et al. (2011) reported that 9% and 13% of 83 female premenopausal nurses working full-time rotating shifts had a deficient or inadequate vitamin D status after summer/autumn and winter/spring, respectively. Important determinants of vitamin D levels were vitamin D supplement use, use of tanning beds, and season. A rapid, forward rotating work shift roster, including 2–9 nights per month, had little effect on serum vitamin D levels in 67 German nurses (Lehnert et al., 2018). The same result was reported in a smaller Japanese study comparing 14 male workers on a rotating shift schedule including nights (1 morning, 1 afternoon, 1 night, 2 rest days), a rotating shift schedule not including nights, or day shift (Itoh et al., 2011). In a Jordanian study of 140 employees, the mean level of 25-hydroxyvitamin D was 23.8 ng/mL. [The Working Group noted that this would suggest that a substantial portion of this population sample had a level below that affecting bone metabolism, despite living in a middle-eastern country at a temperate latitude.] Levels were lower in female, but not male, night shift workers working at least 4 nights (16:00–07:00) per month for 3 years or more (Alefishat & Abu Farha, 2016). In an Italian study of 96 night shift factory workers engaged in a rapid forward rotating shift schedule (1 morning, 1 afternoon, 1 night, 2 rest days) including 2–3 night shifts per week, mean vitamin D levels were lower than

Table 4.18 Clock gene disruption in response to alterations in the light–dark schedule, mapped to selected key characteristics of carcinogens in experimental systems

Key characteristic	Experimental system	Exposure	Clock gene(s) affected	Relevant finding(s)	Reference
Is immunosuppressive	F344 rat (M) Injected at CT 19 with MADB106 tumour cells	Control: LD12:12 Treatments: 6-h advances in the light–dark schedule every 2 d for a total of 10 advances Other rats, used to assess alteration of circadian expression of cytolytic factors, cytokines, and cytolytic capabilities in NK cells, were killed at six time-points 5–7 d into total darkness, corresponding to CT 3, 7, 11, 19, and 23	Total darkness: ↓ *Per2* and *Bmal1* expression and altered cyclicity in NK cells	Advances in the light–dark schedule altered circadian expression and/or ↓ peak expression of *IFNγ*, perforin, and granzyme B in enriched NK cells, with similar changes in NK cell cytotoxicity and ↓ cytolytic activity; ↑ frequency and prevalence of lung tumours at 6–8 wk; no change in plasma corticosterone levels when measured at CT 7 and CT 19	Logan et al. (2012); see also Table 4.5
Is immunosuppressive	Wistar rat (M)	Control: LD12:12 Treatments: 6-h advance in the light–dark schedule alternated with 6-h delay in the light–dark schedule; one advance or delay every 5 d	Advance in the light–dark schedule: WAT expression of *Bmal1* ↑ and *Per2* ↓	↑ WAT expression of the inflammatory marker Inos; ↑ ER marker Pdi	Herrero et al. (2015)
Induces chronic inflammation	*Per2*^{Luc} knock-in mice (M, F)	Control: LD12:12 Treatments: shift to LD18:6, 1×/wk for 4 wk Intraperitoneal injection of LPS at 12.5 mg/kg bw	*Per2* knock-in	Hypothermia and reduced survival after treatment with LPS with 4 wk of treatment; ↑ serum levels of IL1β, GM-CSF, IL12, and IL13	Castanon-Cervantes et al. (2010); see also Table 4.8
Induces chronic inflammation	*Per2*^{Luc} knock-in mice (M, F)	Control: LD12:12 Treatments: advance in the light–dark schedule by 12 h every 5 d for up to 10 wk	*Per2* knock-in	Altered Per2 cyclicity; ↑ mature macrophages (F4/80^{+} CD11b^{+} cells) and pro-inflammatory M1 macrophages (F4/80^{+} CD11b^{+} CD11c^{+} CD206^{-} cells) in adipose tissue; ↓ anti-inflammatory M2 macrophages in adipose tissue; ↑ *IL1β*, *IL6*, and *TNFα* mRNA levels in adipose tissues	Kim et al. (2018); see also Section 4.1.3(b)

Table 4.18 (continued)

Key characteristic	Experimental system	Exposure	Clock gene(s) affected	Relevant finding(s)	Reference
Modulates receptor-mediated effects	Wistar rat (M)	Control: LD12:12 Treatments: 6-h advance in the light–dark schedule alternated with 6-h delay in the light–dark schedule; one advance or delay every 5 d	Advance in the light–dark schedule: hepatic expression of *Bmal1* ↑ and *Per2* and *Rev-erbα* ↓	↓ Hepatic expression of *Pparα* and *Pparγ*	Herrero et al. (2015)
Alters cell proliferation, cell death, or nutrient supply	*K-ras*$^{LSL-G12D/+}$;*p53*$^{flox/flox}$ or *K-ras*$^{LSL-G12D/+}$ mice Induced lung tumour model	Control: LD12:12 Treatment: advance in the light–dark schedule by 8 h for 13 wk	*Per2* or *Bmal1* knockout	Promoted lung tumour growth and progression, ↑ c-Myc expression, enhanced cell proliferation	Papagiannakopoulos et al. (2016)
Alters cell proliferation, cell death, or nutrient supply	B6D2F$_1$ mice (M) inoculated with Glasgow osteosarcoma	Control: LD12:12 Treatment: advance in the light–dark schedule by 8 h for 10 d	Hepatic expression of *Cry1*, *Bmal1*, *Per2*, and *Rev-erb* ↓	Expression of ↓ p53 and ↑ c-Myc; promoted hepatic tumour growth	Filipski et al. (2005)
Alters cell proliferation, cell death, or nutrient supply	B6D2F$_1$ mice with ablated SCN inoculated with Glasgow osteosarcoma	Control: LD12:12 Treatment: advance in the light–dark schedule by 8 h for 10 d	Hepatic and tumour expression of *Per2* and *Rev-erbα* ↓	Promoted hepatic tumour growth	Filipski et al. (2006)

↑, increase; ↓, decrease; bw, body weight; CT, circadian time; d, day; ER, endoplasmic reticulum; F, female; GM-CSF, granulocyte macrophage colony stimulating factor; h, hour; IL, interleukin; Inos, inducible nitric oxide synthase; LD, light–dark schedule, light(h):darkness(h); LPS, lipopolysaccharide; M, male; MADB, mammary adenocarcinoma B cells; mo, month; mRNA, messenger RNA; NK, natural killer; Pdi, protein disulfide isomerase; *Ppar*; peroxisome proliferator activated receptor; *Rev-erb,* circadian nuclear receptor gene; SCN, suprachiasmatic nuclei; TNFα, tumour necrosis factor alpha; WAT, white adipose tissue; wk, week.

Table 4.19 Serum vitamin D levels in shift workers

Location, setting, study design	Exposure level, and number of exposed cases	Response (significance)	Covariates controlled	Reference
Germany Health-care workers Cross-sectional	47 female forward RS (early, late, NS, including 2–9 nights/mo), 20 female DS	–	Age, season, BMI, smoking status, physical activity	Lehnert et al. (2018)
Japan Metal tool factory workers Cross-sectional	14 male workers: 4 in rapid forward RS, 4 in RS not including nights, and 6 DS	–	Age	Itoh et al. (2011)
Jordan University hospital and non-medical staff Cross-sectional	82 NS (≥ 4 nights/mo for ≥ 3 yr), 58 DS, both sexes	NS < DS ($P = 0.003$)	None	Alefishat & Abu Farha (2016)
Italy Aluminium product-manufacturing plant Cross-sectional	96 NS (working nocturnal hours at least 2×/wk), 100 DS	NS < DS ($P < 0.001$)	Age, smoking status, BMI, waist circumference	Romano et al. (2015)

–, not significant; BMI, body mass index; DS, day shift; mo, month; NS, night shift; RS, rotating shift; wk, week; yr, year.

in those of 100 day workers (13.4 ± 5.3 ng/mL vs 21.9 ± 10.7 ng/mL; $P < 0.001$) (Romano et al., 2015). [The Working Group noted that vitamin D has demonstrated protective effects against cancer development in laboratory animal studies, but findings have been inconsistent in studies in humans; a reduced risk of cancer of the colon or rectum and cancer of the bladder, a higher risk of cancer of the prostate and possibly cancer of the pancreas, and no clear association for most other organ sites have been reported (Mondul et al., 2017).]

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