Original research

Melatonin supplementation and oxidative DNA damage repair capacity among night shift workers: a randomised placebo-controlled trial

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Received 29 August 2024
Accepted 8 January 2025

ABSTRACT

Objectives A decreased ability to repair oxidative DNA damage, due to melatonin suppression, is a compelling mechanism by which night shift workers are at an increased risk of cancer. We sought to determine if melatonin supplementation would improve oxidative DNA damage repair among night shift workers.

Methods We conducted a parallel-arm randomised placebo-controlled trial of melatonin supplementation among 40 night shift workers. Supplements were consumed before engaging in day sleep over a 4-week period. All urine excreted during a representative day sleep and night work period before and during the intervention period was collected for measurement of creatinine-adjusted 8-hydroxy-2′-deoxyguanosine (8-OH-dG) as an indicator of oxidative DNA damage repair capacity, with higher concentrations indicating better repair. Linear regression models were used to analyse the association between ln-transformed 8-OH-dG concentration and intervention status during day sleep and night work.

Results The melatonin intervention was associated with a borderline statistically significant 1.8-fold increase in urinary 8-OH-dG excretion during day sleep (95% CI 1.0, 3.2, p=0.06). No statistically significant difference in 8-OH-dG excretion was observed during the subsequent night shift (melatonin vs placebo excretion ratio=0.9; 95% CI 0.6, 1.5; p=0.7).

Conclusions Our results suggest that melatonin supplementation improves oxidative DNA damage repair capacity among night shift workers. Future larger-scale trials are needed to evaluate the impact of varying doses of melatonin supplements and examine the impacts of longer-term use of melatonin supplements by night shift workers.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Normal night-time secretion of melatonin is suppressed among night shift workers. Reduced circulating levels of melatonin may contribute to a decreased ability to repair oxidative DNA damage, thereby putting night shift workers at increased risk of developing cancer.

WHAT THIS STUDY ADDS

⇒ In a 1-month randomised placebo-controlled trial, we demonstrated that consumption of a 3 mg melatonin supplement before engaging in daytime sleep improved the ability of night shift workers to repair oxidative DNA damage. No previous trials of melatonin supplementation and oxidative DNA damage have been conducted.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our findings warrant future larger-scale studies that examine varying doses of melatonin supplements and longer-term impacts of melatonin use. Pending the outcome of such studies, melatonin supplementation may prove to be a viable intervention strategy to reduce the burden of cancer among night shift workers.

INTRODUCTION

Working at night disrupts the circadian system that coordinates our biology with daily light and dark cycles[1] and has been linked to multiple chronic health effects, including increased risks of cancer.[2] Disruption by exposure to light of the secretion of melatonin, a pleiotropic hormone responsible for synchronising circadian rhythms,[3] may factor into the development of cancer among night shift workers. Reactive oxygen species (ROS) produced through cellular metabolism can cause DNA damage, thereby contributing to the pathogenesis of cancer,[4] and melatonin has direct and indirect antioxidant properties, including scavenging of ROS and stimulation of antioxidative enzymes.[5]

8-hydroxy-2′-deoxyguanosine (8-OH-dG) is the most commonly occurring DNA lesion induced by ROS.[6] The majority of 8-OH-dG is excised from DNA by cellular repair machinery and excreted into urine where it can be quantified. This means that better DNA repair capacity leads to higher concentrations of 8-OH-dG in urine. In previous observational studies of night shift workers, we observed that they excreted significantly lower levels of 8-OH-dG during their day sleep and night work periods as compared with night sleep periods, on their days off, suggesting a decreased ability to effectively identify and excise the lesions during day sleep and night work.[7 8] We also observed that decreased 8-OH-dG levels were associated with

To cite: Zanif U, Lai AS, Parks J, *et al*. *Occup Environ Med* Epub ahead of print: [please include Day Month Year]. doi:10.1136/oemed-2024-109824

decreased circulating melatonin levels during both day sleep and night work, which is consistent with melatonin's previously identified role in upregulating DNA repair machinery involved in the repair of 8-OH-dG lesions.[9 10]

Based on our previous observations, we launched a randomised placebo-controlled trial of oral melatonin supplementation among night shift workers in Canada to evaluate impacts on 8-OH-dG excretion as a measure of oxidative DNA damage repair capacity. We also explored the impacts of melatonin supplementation on daytime sleep measures and subjective measures of sleepiness during night shift work.

METHODS

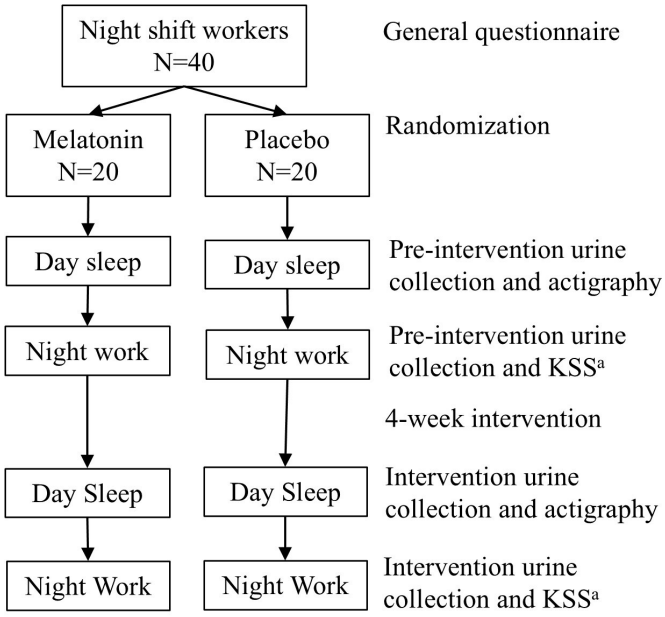
Since Health Canada has approved oral melatonin for use at bedtime in adults with altered sleep schedules (eg, night shift workers), the proposed study qualified as a phase IV trial requiring no additional regulatory approvals.[11] The study was registered with ClinicalTrials.gov (NCT03945955).

Study participants

Participants were recruited via email advertisements sent by union representatives to union members belonging to the British Columbia Federation of Labour. Referrals from eligible/ineligible participants were also used to identify potential participants for the study. Eligibility criteria included (1) living and working in the Greater Vancouver area; (2) being between the ages of 18 and 50; (3) having a body mass index (BMI) between 18.5 and 30 kg/m^2; (4) working at least two consecutive night shifts per week (≥7 hours per night ending no earlier than 06:00) for at least 6 months; (5) sleeping, on average, 6 hours during day sleep periods (ie, main period of sleep during daylight hours after completing a night shift); (6) not currently using marijuana or illicit drugs; (7) no personal history of sleep disorders, hormone disorders, seizure disorders or chronic medical conditions (eg, cancer, diabetes, kidney disease, liver disease, asthma, cardiovascular disease and infectious disease); (8) not currently pregnant or currently breastfeeding; (9) no trans-meridian travel within 4 weeks of data collection and (10) not currently using melatonin supplements. Our goal was to recruit 40 participants based on power calculations using data from our previous observational studies.[7 8]

Study design

Participants were randomised (coin-tossing blocked on sex) by the project manager (ASL) into parallel placebo and melatonin supplement intervention arms (1:1), and, along with field staff, were blinded to intervention status (figure 1). Participants were instructed to consume the supplements with a meal, after completing their night shift, and 1 hour before their intended day sleep bedtime, as per Health Canada guidelines, over a 4-week period.[11] In addition to a general questionnaire completed at the time of recruitment, data and biospecimens were collected during the second of two subsequent day sleep and night work periods, once before commencing the intervention and once near the end of the 4-week intervention period (figure 1). Participants wore wrist actigraphs to collect measures of sleep during the day sleep period. Participants also collected all urine excreted during the day sleep and subsequent night shift periods (separate containers for day sleep and night shift). Participants were instructed to void their bladders prior to each urine collection period and collect only the subsequent urine that was excreted, including the first void immediately after the period ended.



[a]Karolinska Sleepiness Score

Figure 1 Outline of placebo-controlled trial of melatonin supplementation among night shift workers. Data and urine samples were collected during a day sleep and a night work period before commencing the intervention (ie, consuming melatonin/placebo supplements) and during a day sleep and night work period near the end of the 4-week intervention.

Supplement formulation

A dose of 3 mg of melatonin was selected for the study because it would likely produce physiological to supraphysiological levels of circulating melatonin in most people even after accounting for interindividual variation in bioavailability of melatonin.[12–14] The melatonin supplements and identical-looking placebo supplements were formulated in accordance with requirements described in Health Canada's National Health Product Directorate Quality of Natural Health Products Guide[15] and the Natural Health Products Ingredient Database[16] by Macdonald's Prescriptions (Vancouver, BC). Melatonin capsules were compounded using analytical grade melatonin with microcrystalline cellulose filler, and placebo capsules only contained filler. Unused melatonin and placebo supplements at the end of the intervention were collected and counted.

Questionnaire

A general study questionnaire was administered face to face by staff at the time of enrolment. In addition to height and weight for calculation of BMI, the questionnaire collected data on demographic factors (date of birth, biological sex, gender, ethnicity and education), lifestyle factors (smoking status, frequency of alcohol consumption) and job information (industry of employment, shift schedule and years in current job).

Actigraphy and sleepiness

Participants wore an actigraph (Spectrum Plus, Koninklijke Philips NV) on their non-dominant wrist during 2 daytime sleep periods, one before the intervention and one near the end of the intervention. The actigraph recorded activity in 2 min epochs during the sleep periods. Participants kept a sleep log to record bed and wake times as well as any awakenings that occurred during the sleep periods. These data were used to score the

actigraphy data and estimate daytime sleep duration as well as wake after sleep onset (WASO, ie, amount of time a person is awake after falling asleep and before waking up). During the subsequent night shift periods, participants were asked to complete the Karolinska Sleepiness Scale (KSS), which asked them to indicate their degree of sleepiness on a 9-point scale (higher values indicate greater sleepiness) in the past 5 min.[17] Participants were instructed to complete the KSS towards the end of their night shifts.

Urine collection

Each participant collected all urine excreted over a total of four separate periods (preintervention day sleep, preintervention night work, intervention day sleep and intervention night work), the same periods for which actigraphy and KSS data were collected (figure 1). Urine was collected in 3.5 L containers, and participants were provided coolers with ice packs to store the containers between urine voids. Participants recorded the time of each urine void and were asked to note any issues with the urine collections (eg, spills and contamination). After the final urine void for a given period, study staff collected the urine sample, which was immediately transported to the laboratory for processing. The mean time between the final urine void and processing of samples was 132 min with no significant difference in time between day sleep and night shift samples. Urine in the sample containers was mixed by swirling/inversion of the containers before aliquoting into five 3.6 mL cryotubes. Samples were stored at −80°C until ready for assay.

Assays

The Exposure Biology and Analytical Chemistry Laboratory at Duke University used high pressure liquid chromatography-tandem mass spectrometry to measure 8-OH-dG and 6-sulfatoxymelatonin (aMT6s), a marker of circulating melatonin levels, in each urine sample.[18 19] A second set of urine samples from a randomly selected 10% of participants were dispersed among study samples as blind duplicates. Values below the limit of detection (LOD) were replaced with LOD/√2.[20] Creatinine was measured in the urine samples with the Creatinine Colorimetric Assay Kit (Cayman Chemical) and a microplate reader. Concentrations of 8-OH-dG and aMT6s were creatinine adjusted for all analyses and expressed as ng/mg-creatinine concentrations.

Statistical analysis

Linear regression models were used to analyse the association between 8-OH-dG concentration, as measured during the intervention period, and intervention status (melatonin vs placebo). Based on the distribution of the data, 8-OH-dG concentrations were ln-transformed in the regression analyses. The regression models included ln-transformed preintervention 8-OH-dG concentration as a covariate. The regression point estimate for intervention status was exponentiated, generating an estimate of the ratio of 8-OH-dG concentrations between the melatonin and placebo arms. Statistical significance was assessed as $p \leq 0.05$.

In additional analyses, sleep duration, WASO and KSS measured during the intervention period were evaluated in association with intervention status using linear regression models. Preintervention sleep duration, WASO and night shift KSS were included as covariates in the respective models. Sleep duration, WASO and KSS data were ln-transformed for the analyses.

RESULTS

Recruitment and data collection occurred between June 2022 and October 2023. A total of 190 people were assessed for eligibility, with 84 (44%) identified as eligible. Of the 84 individuals, 41 (49%) agreed to participate in the study. One of the 41 participants dropped out after completing preintervention data and biospecimen collection. This participant was excluded from all analyses. Of the 40 remaining participants, one randomised to the placebo arm was unable to collect preintervention urine or data during the night shift period, meaning that their data only contributed to the day sleep analyses.

Table 1 Characteristics of study participants stratified by intervention status

	Intervention	
	Melatonin	Placebo
Characteristic	n, mean (%, SD)	n, mean (%, SD)
Sex/gender		
Female	12 (60)	12 (60)
Male	8 (40)	8 (40)
Age	36.1 (7.8)	33.0 (6.3)
Ethnicity		
East Asian	6 (30)	5 (25)
White	6 (30)	4 (20)
Other	8 (40)	11 (55)
Education		
High school or diploma/certificate	<4*	4 (20)
Bachelor's or graduate degree	>16*	16 (80)
Industry of employment		
Healthcare and social assistance	>16*	>16*
Other	<4*	<4*
Months at current job	69.0 (61.0)	49.4 (46.2)
Shift schedule		
2 days, 2 nights, 4 off	7 (35)	10 (50)
Other	13 (65)	10 (50)
Current smoker		
Yes	0 (0)	<4*
No	20 (100)	>16*
Alcohol consumption frequency		
≥once per week	11 (55)	5 (25)
Never to three times per month	9 (45)	15 (75)
Body mass index (kg/m^2)		
18.5–24.9	9 (45)	10 (50)
25.0–29.9	11 (55)	10 (50)

*Exact numbers are not displayed due to small numbers (<4) in one category of the variable.

Characteristics of the 40 participants included in the analyses are summarised in table 1. All participants self-reported as cisgender. 60% of participants in the melatonin and placebo arms were female. Most participants reported being of non-East Asian and non-white ancestry (40% melatonin arm and 50% placebo arm), and most reported completing a bachelor's or graduate degree as their highest attained education level (80% placebo arm; melatonin arm number suppressed due to small cell count). Most participants in both arms worked in the healthcare and social assistance industry (numbers suppressed due to small cell counts). Participants in the melatonin arm tended to be at their current jobs for a longer duration than those in the placebo arm (mean (SD): 69.0 (61) vs 49.4 (46.2) months, respectively). 35% and 50% of participants in the melatonin and placebo arms, respectively, worked a schedule rotating between two day shifts, two night shifts and four days off. The remaining participants worked various different schedules involving 3–7

Table 2 Descriptive statistics for 6-sulfatoxymelatonin concentration, 8-hydroxy-2′-deoxyguanosine concentration, sleep duration, wake after sleep onset and Karolinska Sleepiness Scale measured preintervention and during intervention for melatonin and placebo intervention arms during day sleep and/or night work

Measure	Preintervention/intervention period	Melatonin/placebo	Day sleep/night shift	n	Mean	SD	Median
6-sulfatoxymelatonin (aMT6s) (ng/mg)	Preintervention	Melatonin	Day sleep	20	7.0	25.0	1.2
			Night shift	20	3.0	2.6	2.0
		Placebo	Day sleep	20	6.2	18.0	0.9
			Night shift	19	2.2	3.1	1.3
	Intervention	Melatonin	Day sleep	20	171.1	192.7	90.8
			Night shift	20	5.2	4.6	3.3
		Placebo	Day sleep	20	1.9	1.8	1.5
			Night shift	20	2.3	2.4	1.3
8-hydroxy-2′-deoxyguanosine (8-OH-dG) (ng/mg)	Preintervention	Melatonin	Day sleep	20	1.7	1.1	1.5
			Night shift	20	2.7	3.6	1.3
		Placebo	Day sleep	20	1.9	2.0	1.1
			Night shift	19	2.1	2.7	1.1
	Intervention	Melatonin	Day sleep	20	3.6	4.8	1.9
			Night shift	20	2.3	1.8	1.9
		Placebo	Day sleep	20	1.7	1.8	1.4
			Night shift	20	2.6	2.5	1.9
Sleep duration (min)	Preintervention	Melatonin	Day sleep	16	280.6	131.3	270.0
		Placebo	Day sleep	15	248.1	89.7	260.0
	Intervention	Melatonin	Day sleep	17	222.1	88.3	254.0
		Placebo	Day sleep	18	233.6	73.8	218.0
Wake after sleep onset (min)	Preintervention	Melatonin	Day sleep	16	47.1	25.9	43.0
		Placebo	Day sleep	15	21.4	12.1	18.0
	Intervention	Melatonin	Day sleep	17	37.6	24.8	33.0
		Placebo	Day sleep	18	35.5	30.6	32.0
Karolinska Sleepiness Scale	Preintervention	Melatonin	Night shift	20	5.2	2.3	6.0
		Placebo	Night shift	19	5.7	1.6	6.0
	Intervention	Melatonin	Night shift	20	4.9	2.1	6.0
		Placebo	Night shift	20	5.9	1.6	6.0

night shifts per week. No individuals in the melatonin arm and very few individuals in the placebo arm (number suppressed due to small cell counts) reported being current smokers. A greater proportion of the melatonin arm as compared with the placebo arm tended to consume alcohol on one or more occasion each week (55% vs 25%).

Of the 175 urine samples sent for assay, 16 had 8-OH-dG and aMT6s levels that were below the LOD. Coefficients of variation (CVs) were calculated for aMT6s and 8-OH-dG from the blind duplicate data. The CVs for aMT6s and 8-OH-dG were 21% and 33%, respectively. No participants reported any issues with urine sample collection. Counts of unused pills collected at the end of the intervention period indicated that none of the participants missed any of their supplement doses.

Based on the descriptive statistics presented in table 2, aMT6s concentration data were right-skewed (eg, SD≥mean). Though individual levels were quite variable, overall, the melatonin intervention produced circulating melatonin levels during day sleep (median=90.8 ng/mg) that were an order of magnitude higher than day sleep levels in the placebo arm (median=1.5 ng/mg). Two participants in the intervention arm had day sleep circulating melatonin levels that were lower than the median level in the placebo arm (1.5 ng/mg). Circulating melatonin levels for the melatonin intervention arm dropped significantly during the subsequent night shift (median=3.3 ng/mg) but remained slightly greater than circulating melatonin levels among the placebo arm during the night shift (median=1.3 ng/mg). 8-OH-dG concentrations also appeared to be right-skewed (table 2). Median levels ranged from 1.1 ng/mg in the preintervention placebo group during both day sleep and night shift work to 1.9 ng/mg during the intervention period in the melatonin group during both day sleep and night shift work and in the placebo group during night shift work.

Daytime sleep actigraphy data were missing for nine participants from the preintervention period and five participants from the intervention period (table 2). Reasons for missing sleep data included actigraph malfunction and removal of the actigraph by the participant during the day sleep period. Mean and median sleep durations tended to be lower during the intervention as compared with the preintervention period for both the melatonin and placebo groups. For example, median sleep duration for the melatonin group during the intervention was 254 min but was 270 min before the intervention (table 2). Mean and median WASO were higher in the melatonin group during the preintervention period as compared with the intervention period (eg, median WASO 43.0 vs 33.0 min). For the placebo group, mean and median WASO were lower during the preintervention period as compared with the intervention period (eg, median WASO 18.0 vs 32.0 min) (table 2). Mean (SD) KSS varied from 4.9 (2.1) for the melatonin group during the intervention to 5.7 (1.6) for the placebo group during preintervention. Median KSS was equal to 6.0 across both arms during the preintervention and intervention periods (table 2).

Table 3 Impact of melatonin versus placebo intervention on 8-hydroxy-2′-deoxyguanosine, sleep duration, wake after sleep onset and Karolinska Sleepiness Scale

Outcome	Day sleep/night shift	n	Intervention effect*	95% CI	P value
8-hydroxy-2′-deoxyguanosine (8-OH-dG)	Day sleep	40	1.8	1.0, 3.2	0.06
	Night shift	39	0.9	0.6, 1.5	0.7
Sleep duration	Day sleep	29	0.8	0.6, 1.1	0.2
Wake after sleep onset	Day sleep	29	0.5	0.2, 1.0	0.07
Karolinska Sleepiness Scale (KSS)	Night shift	39	0.9	0.7, 1.1	0.3

*Ratios of measures (8-OH-dG concentration, sleep duration, wake after sleep onset and KSS) comparing melatonin versus placebo intervention groups (eg, 1.8-fold higher urinary 8-OH-dG level in melatonin arm as compared with placebo arm). Generated from linear models of ln-transformed measures during intervention period regressed on intervention status and ln-transformed preintervention measures.

Compared with placebo, melatonin supplementation was associated with a borderline statistically significant 1.8-fold increase in urinary 8-OH-dG excretion during day sleep (95% CI 1.0, 3.2, p=0.06) (table 3). Inclusion of duration at current job and alcohol consumption frequency as covariates had a minimal impact on the intervention point estimate (<10% difference; results not shown). No statistically significant difference in 8-OH-dG excretion was observed during the subsequent night shift (melatonin vs placebo excretion ratio=0.9; 95% CI 0.6, 1.5; p=0.7).

As compared with placebo, melatonin supplementation was associated with a 0.8-fold decreased sleep duration, but the effect was not statistically significant (95% CI 0.6, 1.1; p=0.2). As compared with placebo, melatonin supplementation was associated with a 0.5-fold decrease in WASO, and the effect was of borderline statistical significance (95% CI 0.2, 1.0; p=0.07). No statistically significant difference in KSS between the melatonin and placebo groups was observed during night shift work (melatonin vs placebo KSS ratio=0.9; 95% CI 0.7, 1.1; p=0.3).

In exploratory analyses, when excluding the two participants in the melatonin intervention arm with particularly low day sleep aMT6s levels during the intervention (<1.5 ng/mg), a statistically significant 1.9-fold increase in urinary 8-OH-dG excretion during day sleep was observed (95% CI 1.0, 3.4; p=0.05). When excluding five participants in the melatonin intervention arm with the lowest day sleep aMT6s levels (≤30 ng/mg creatinine), a statistically significant 2.0-fold increase in urinary 8-OH-dG excretion during day sleep was observed (95% CI 1.1, 3.4; p=0.04). Exclusion of these participants had no material impact on the results of the night shift 8-OH-dG, sleep duration, WASO or KSS analyses (results not shown).

DISCUSSION

In a randomised placebo-controlled trial, we observed that a 3 mg melatonin supplement consumed by night shift workers before engaging in daytime sleep, which, on average, produced a large increase in circulating melatonin levels, led to a borderline statistically significant 80% increase in urinary excretion of 8-OH-dG during the daytime sleep period. During the subsequent night shift, circulating melatonin levels remained only slightly elevated with no significant difference in urinary excretion of 8-OH-dG. These findings are consistent with our previous observational studies in which night shift workers during their day sleep and night work periods, when they had low circulating levels of melatonin, excreted significantly less 8-OH-dG in urine relative to their night sleep periods on their days off when they had much higher circulating levels of melatonin.[7 8]

Melatonin has been shown to upregulate expression of genes belonging to the nucleotide excision repair (NER) pathway,[9 10] which contributes to the excision and repair of 8-OH-dG lesions.[21] Findings from our previous studies suggested that low levels of melatonin during day sleep and night work among night shift workers lead to a reduction in the ability of the NER pathway to recognise and/or excise 8-OH-dG lesions.[7 8] Such an effect would be expected to cause an accumulation of oxidative DNA damage in the tissues of night shift workers. Though oxidative DNA damage and the role of melatonin were not specifically evaluated, a previous study observed significantly increased levels of unrepaired DNA damage in leukocytes, as measured by the alkaline comet assay, when comparing participants on a simulated night shift schedule to those on a simulated day shift schedule at a sleep laboratory.[22] Findings from our trial suggest that melatonin supplementation during day sleep may help mitigate the negative impacts of night shift work on oxidative DNA damage repair.

Overall, the 3 mg melatonin supplement produced supraphysiologic levels of circulating melatonin among night shift workers during their day sleep period. In the trial, we observed a mean (SD) aMT6s level of 171.1 ng/mg (192.7) after consumption of the melatonin supplement while, in a previous study, we observed day shift workers to have a mean (SD) aMT6s level of 32.6 ng/mg (17.9) during a regular night of sleep without any melatonin supplementation.[7] Circulating melatonin levels among participants in the melatonin arm of the trial were quite variable, however. When excluding participants with the lowest day sleep aMT6s levels measured in the melatonin arm during the intervention period, an increase in urinary 8-OH-dG excretion due to the melatonin intervention was observed, and this effect was statistically significant. Large interindividual variation in bioavailability of exogenous melatonin poses a challenge to identifying ideal doses of melatonin supplements for intervention/treatment.[23 24]

Few studies have evaluated the impact of melatonin supplementation on daytime sleep among night shift workers. Overall, the evidence suggests a modest increase in day sleep duration and decreased awakenings.[25 26] While we did find suggestive evidence that the melatonin intervention led to decreased WASO, we observed a 20% decrease in day sleep duration due to the intervention, though the difference was not statistically significant. We also did not observe any significant impact of the intervention on KSS. We did not expect the intervention to have a negative impact on KSS since circulating melatonin only remained slightly elevated during the night work period. In addition, previous research has demonstrated that even high doses of melatonin do not tend to produce sedative effects.[27]

This trial is the first of its kind to evaluate the impact of melatonin supplements on oxidative DNA damage among night shift workers. The comprehensive data collected and generated by the study, including measures of circulating melatonin, the KSS and objectively measured sleep duration and WASO, supported a

detailed evaluation of the impacts of melatonin supplementation among night shift workers.

Because the study was not conducted in a laboratory setting, we could not control for exposure to light. Light exposure is the primary mediator of the negative impacts of night shift work on melatonin secretion,[28] meaning that it is not a confounding factor that would have biased results. Rather, differences in light exposure between participants would have contributed to the variation observed among participants in their circulating melatonin levels. We also did not control for differences in menstrual cycle among female participants. While melatonin has been shown to impact female menstrual cycles,[29] there is no established impact of menstrual cycle on melatonin secretion. As such, we would not expect differences in menstrual cycle to bias our results.

Most participants worked in the Health Care and Social Assistance industry which limits the generalisability of results. While we did not capture other occupational exposures that may contribute to the generation of oxidative DNA damage, given the randomisation of participants, it is unlikely that unmeasured exposures would have biased study results. The small sample size of the trial contributed to imprecise effect estimates, as indicated by the large confidence intervals, and limited our ability to detect modest impacts of the intervention. The limited sample size also did not support the conduct of meaningful stratified analyses (eg, by biological sex). Given the limited scope of the trial, we were unable to evaluate multiple doses of melatonin supplements, nor were we able to evaluate the long-term efficacy of melatonin supplementation.

Increased oxidative DNA damage due to diminished DNA repair capacity is a compelling mechanism that may contribute to the carcinogenicity of night shift work. Our randomised placebo-controlled trial suggested melatonin supplementation may improve oxidative DNA damage repair capacity among night shift workers. Our findings warrant larger-scale trials considering multiple doses of melatonin, interindividual variability in melatonin bioavailability, and the impacts of long-term use of supplements. Assessing long-term efficacy is critical since those who work night shifts for many years would need to consistently consume melatonin supplements over that time frame to maximise the potential cancer prevention benefits.

Contributors PB is the guarantor. PB conceived and oversaw the study. CBM, NA, JJZ and PB contributed to the design of the study. ASL coordinated and supervised fieldwork activities. UZ, JP and AR led the data analysis. XW, YL and JJZ conducted the laboratory assays. UZ, ASL, JP, AR, CBM, NA, XW, YL, JJZ and PB participated in the writing of manuscript.

Funding This research is funded by a Proof-of-Concept Intervention Grant in Primary Prevention of Cancer (Action Grant) of the Canadian Cancer Society (CCS grant #707217) and the Canadian Institutes of Health Research-Institute of Cancer Research in partnership with the Cancer Research Society and the BC Cancer Foundation.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study was approved by the BC Cancer Agency/University of British Columbia Research Ethics Board (H19-00780). Research participants consented to the publication of results from this study.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. Researchers are welcome to contact the corresponding author for data access.

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