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Measuring and identifying large-study metrics for circadian rhythm disruption in female flight attendants

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Objectives Flight attendants can experience circadian rhythm disruption due to travel through multiple time zones. The objectives of this study were to determine whether flight attendants are more likely than teachers (comparison group) to experience circadian disruption, as measured by melatonin production, and to identify metrics of circadian disruption for epidemiologic studies of reproductive health in which biomonitoring is infeasible.

Methods Each day, for one menstrual cycle, 45 flight attendants and 26 teachers kept a daily diary, collected and measured their overnight urine, and wore an activity monitor to assess sleep displacement. The relation between melatonin production and flight attendant and teacher status was analyzed with linear and multiple logistic regression. The relation between sleep displacement, melatonin, and flight-history-derived variables (including time zones crossed) were examined with exploratory factor analyses.

Results Flight attendants experience increased circadian disruption, as measured by a higher adjusted melatonin rate variance, than teachers [2.8×10^5 versus 1.0×10^5 (ng/hour)^2, respectively; P=0.04] and are more likely to be in the highest quartile of melatonin variance (odds ratio 2.3; 95% confidence interval 0.6–9.1). In the factor analysis, the number of time zones crossed was related to both melatonin desynchronization and sleep displacement.

Conclusions Flight attendants experience increased circadian disruption, as measured by more variable melatonin rates, than a minimally flying comparison group. For epidemiologic studies of flight crews in which melatonin measurement is infeasible, the number of time zones crossed is a useful indicator of both sleep displacement and melatonin desynchronization.

Key words aerospace medicine, circadian rhythm, jet lag syndrome, melatonin, sleep disorders circadian rhythm, work schedule tolerance.

Air travel during normal sleep hours and multiple time-zone changes are an integral part of the workplace for many of the 198 000 air crew members employed in the United States, including about 113 700 flight attendants (1). The issues concerning the disruption of flight attendants' sleep cycle and circadian rhythm may in some ways resemble those of shift workers, but a flight attendant's work schedule often lacks the regularity that assists a shift worker's circadian resynchronization. In addition, flight attendant's work often involves rapid movement through multiple time zones in directions known to maximize circadian disruptions, and the resultant disruption of zeitgeibers (external time cues) is different than for shift workers. Because circadian disruption may affect the hormonal balance requisite to reproductive health, the National Institute for Occupational Safety and Health (NIOSH) is currently conducting reproductive health studies among flight attendants. Circadian disruption has been described for small numbers of flight attendants or for flight crews on specific flights (2–5), but no methods exist to assess the overall circadian disruption of larger groups of these workers.

The effect of travel across multiple time zones, or "jet lag", has three major components: external desynchronization, or the disparity between external time cues and internal physiological rhythms;

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internal desynchronization, or the disruption of internal physiological rhythms relative to each other; and sleep loss resultant from internal and external desynchronization (6). The time required for resynchronization after time-zone changes depends on the flight direction (eastward flights result in greater disruption), the number of time zones crossed, and individual factors including age and intrinsic circadian characteristics (7, 8). Resynchronization after flights over multiple time zones takes 4–6 days for many monitored functions, including sleep and cortisol level (2, 3), but, for some hormones and for people who readjust with more difficulty, resynchronization requirements of 10–12 days or more have been reported (7, 4). In studies of air crew crossing multiple time zones, initial sleep changes appear to be due to sleep loss on the outward journey, followed by sleep changes induced by circadian desynchronization (5). This apparent interrelatedness of sleep disruption and desynchronization is plausible, since both the human circadian pacemaker and a separate sleep homeostat interact to affect the sleep-wake cycle (9).

The biomarkers used to study desynchronization include melatonin, the major hormone produced by the pineal gland. Its well-characterized rhythm is controlled endogenously by a light-entrained circadian pacemaker—levels rise at night and fall during the day (10). Melatonin levels have been measured in shift workers to characterize their circadian rhythm (11–13), although these measurements have typically been made in a sleep laboratory or collected at frequent intervals not feasible for field studies.

In 1995, we conducted a biomonitoring feasibility study of 45 flight attendants and 26 teachers to determine whether daily urine and saliva samples for reproductive hormones, melatonin, and cotinine, as well as wrist activity data to measure sleep displacement, could be collected successfully for a highly mobile working population. Teachers were selected as a comparison group for this reproductive health study primarily because this predominantly female occupation has minimal air travel, few reproductive hazards, and comparability to flight attendants on several key demographic characteristics (eg, age, race, education, parity) (14). Prior analyses of data from this study estimated the cosmic radiation dose for flight attendants (15) and showed that flight attendants incurred significantly similar sleep impairment when compared with teachers (16).

The purpose of this study was to determine whether flight attendants are more likely than teachers to experience circadian disruption, as measured by melatonin production. A second objective was to identify metrics of circadian disruption (from multiple measures of sleep, activity, and flight history) that could be used in large epidemiologic studies in which biomonitoring is infeasible.

Participants and methods

The study methods have been described previously (14–16) and are summarized in this report.

Study population and data collection

The study protocol was approved by the NIOSH Human Subjects Review Board, and signed informed consent was obtained from all the participants. Two major airline companies were selected for this study, one with a domicile (hub or home base) in Miami and the other with a domicile in Seattle. These domiciles were chosen to enroll flight attendants who flew a greater-than-average number of north-south routes in order to maximize the ability to separate analytically the two primary exposures of interest (cosmic radiation and circadian disruption). Because normal work schedules were not altered for this study (15), however, east-west routes were also flown by the participants. In the United States, there is tremendous diversity and flexibility in the work schedules of flight attendants, who schedule work flights to fit their life-styles. In addition, many flight attendants are on “reserve” status; in other words, the flight attendant’s working flight schedule is unselected and unknown until shortly before the flight is flown. Because the design was an observational study of working flight attendants flying their normal schedules rather than an experiment, additional criteria for routes or schedules flown were not applied. Altogether 45 flight attendants and 26 teachers were enrolled from company and union rosters in the two cities. The eligibility criteria were as follows: (i) current full-time employment as a flight attendant or teacher, (ii) age 18–45 years, (iii) not currently pregnant, (iv) had not yet reached menopause, (v) not using an intrauterine device (IUD), (vi) not using oral contraceptives or other exogenous hormones, and (vii) not planning to use an IUD or exogenous hormones in the next three months. An interviewer-administered baseline questionnaire included questions on demographics, medical history, life-style factors, reproductive history, and occupational factors. A separate self-administered questionnaire collected information on work environment stressors, and acute and chronic stress. A daily diary modeled after that of Gold et al (17) included questions about urine collection, sleep quality, acute illnesses and medications used, active and passive smoking, alcohol consumption, caffeine consumption, exercise, ergonomic stressors, psychosocial stress, and dieting.

Urine and actigraphy

Daily first morning urine, pooled with any urine voided during the night, was measured, and 5-milliliter samples were collected and retained without a preservative in the woman's home refrigerator during the biomonitoring period (usually one menstrual cycle). Transport procedures were developed to allow flight attendants to carry samples and collection materials as they traveled. Sample quality was not impaired or diminished by work-related travel (14).

References to "melatonin" in this study refer to melatonin as measured by its urinary metabolite, 6-sulfatoxymelatonin (6SMT), assayed by the direct radioimmunoassay method of Bojkowski et al (18) (Elias USA, Inc, Osceola, WI, USA). 6SMT has been shown to correlate with plasma melatonin levels, it appears to be produced at a stable rate within individuals, and it has been used as a circadian rhythm biomarker (18). The 6SMT data were judged acceptable after review of the quality assurance information from the laboratory. For an additional series of 25 duplicate samples from teachers, coefficients of variation were less than 15% for 22 (88%) of the samples. Wrist activity (actigraph) and diary data were used to calculate the mean hourly rate of overnight 6SMT production (for the hours since the last urination before the sleep period) in nanograms/hour. Three metrics for melatonin production were created from 6SMT data (table 1). In addition to each woman's mean overnight 6SMT rate and within-woman variance, an index of low melatonin production (percentage of low melatonin) was constructed. Nighttime melatonin production is consistent for and has little day-to-day variability within an undisrupted person. We based the index on

Table 1. Measures and indicators of circadian rhythm disruption. [6SMT = 6-sulfatoxymelatonin, HPP = home phase proportion, MSP = main sleep period (which can occur at any time during each 24-hour period), SSI = standard sleep interval (the time between 2200 and 0800, home base time, "home base time" being the time within the time zone of the domicile in which a flight attendant was based or the city in which a teacher lived)]

Source	Measure or indicator	Definition	Group [a]			
			Flight attendants		Teachers	
			Unadjusted mean	SD	Unadjusted mean	SD
Melatonin production	Rate	Overnight rate of urinary 6SMT production during the biomonitoring period (ng/h) analyzed as the mean in the linear regression and as daily rate in repeated measures analysis	1398	561	1215	689
	Variance [b]	Within-woman variance of 6SMT rate $(ng/h)^2$	2.4×10^5	3	1.3×10^5	4
	Percentage of low melatonin	Proportion of monitoring days for which the rate was less than 50% of the mean of a woman's highest four rate days [c]	0.31	0.06	0.31	0.07
Melatonin collection time	HPP mean	Mean of the proportion of time within the SSI during which sampled urine was produced	0.90	0.12	0.96	0.04
	HPP variance	Within-woman variance of HPP	0.037	0.054	0.009	0.012
Sleep or activity	Sleep efficiency	Proportion of the MSP [d] spent asleep	0.86	0.02	0.87	0.02
	Nocturnal sleep fraction	Proportion of 24-hour sleep time within the SSI	0.84	0.14	0.92	0.04
	Total sleep time in MSP	Total sleep time in the MSP (minutes)	449	48	396	42
	MSP midpoint [d]	Time midpoint of the MSP (minutes from midnight)	225	76	180	45
	Variance of the MSP midpoint [d]	Within-woman variance of the MSP midpoint (minutes ^{2})	62	72	15	8
	Activity acrophase [d]	Time of daily peak of the cosine curve fitted to the rest or activity cycle (minutes from midnight)	921	74	868	46
	Activity acrophase variance [d]	Within-woman variance of the activity acrophase (minutes ^{2})	66	80	20	9
Flight histories [e]	Cumulative number of time zones crossed	Sum of the absolute values of the time zones crossed for each flight	134	111	0	·
	Mean number of time zones crossed/flight	Cumulative number of time zones crossed / total number of flight segments	0.7	0.8	0	·
	Cumulative SSI travel	Cumulative time spent working (flying) within the SSI (minutes)	9088	9794	0	·
	Mean SSI travel/flight	Cumulative SSI travel / total number of flight segments (minutes/flight)	67	95	0	·

[a] N=33–43 flight attendants and 21–25 teachers, based on specific missing or excluded data.
[b] Dependent variable normalized with a natural log transformation prior to the analysis, then untransformed for the reported results. The statistics reported are the geometric means and geometric standard deviations.
[c] Nighttime melatonin production is consistent for and has little day-to-day variability within an undisrupted person. Normalized with arcsin transformation prior to the analysis, then untransformed for the reported results.
[d] The daily midpoint of the MSP and the daily time of maximum activity (activity acrophase) are consistent for and have little day-to-day variability within an undisrupted person.
[e] For the flight attendants, on the basis of 4 months of flight histories and recreational travel records. [See the text for details.] Cumulative figures are yearly estimates. For teachers, these estimates were set to 0 since the median estimated time zones crossed for their minimal air travel was 0.

the hypothesis that decreased overnight rates would indicate disruption (since a decreased rate could indicate either suppression or shifting of the characteristic 24-hour rhythm of melatonin) and compared each woman's melatonin overnight rate to her own data since there are considerable interindividual differences in normal melatonin levels. We calculated the proportion of days at less than 50% of the mean of a woman's highest four melatonin rate days.

To assess departure of the urine collection time period from a standard overnight sleep interval, home phase proportion mean and variance were calculated. This metric describes the proportion of time between 2200 and 0800 home base time during which the sampled urine was produced.

Sleep and wakefulness were estimated from wrist-activity data collected with the Mini Motionlogger Actigraph System [Ambulatory Monitoring Inc (AMI), Ardsley, NY, USA]. Each participant was asked to wear the wristwatch-like actigraph 24 hours a day during the biomonitoring period, removing it for bathing only. The participants were also asked to push the event marker button of the actigraph to electronically mark events including sleep and naps. Artifact removal, sleep estimation, and cosinor analysis were performed with AMI ACT and ACTION3 software. For the flight attendants, actigraph and diary data were converted to home-base time. Sleep and wakefulness were estimated according to the electroencephalographically validated algorithm of Cole et al (19), modified for 3-minute epochs. For these analyses, seven summary measures of disturbance of activity rhythms and estimated sleep were derived for each woman from her daily actigraph data (table 1).

Flight history records

Four of the 16 circadian disruption variables in table 1 derive from the records of the individual flight histories, obtained for each flight attendant from the airlines. Cumulative time zones crossed per year and average time zones crossed per flight segment were estimated for the flight attendants from the flight history and less detailed recreational travel records obtained from the airlines. The flight history records contained the city of origin, destination, date, and departure and arrival times of each flight segment for 4 months during the study period. For the teachers, block (air travel) time and time zones crossed were estimated from questionnaire information describing usual number of time zones per flight, usual time per flight, and number of flights in the preceding 6 months, which included the summer break. The flight attendants traveled an average of 21.5 (SD 9.9) flight segments a month, totaling 54.5 (SD 20.6) block hours a month, including unofficial (commuter and recreational) travel. The teachers reported an average of 10.2 hours of air travel in the preceding 6 months. For the flight attendants, the cumulative and per-flight-segment standard sleep interval travel (proportion of flight segment time flown between 2200 and 0800 home base time) were calculated to assess work-related disruption of the standard sleep interval.

Data analysis

Data on 8 of the 71 women were excluded from the analysis because of withdrawal from the study (N=1), missing melatonin data (N=2), or nonphysiological (abnormally high) melatonin levels (N=5). Two of these women reported taking melatonin supplements during the biomonitoring period. Five individual urine samples from two women were deleted from the analysis because the dates associated with the samples could not be determined. With the exception of the actigraph ACT and ACTION3 software mentioned previously, SAS (20) was used for all statistical procedures.

To assess circadian rhythm disruption among the flight attendants, we derived three continuous measures of melatonin production (melatonin rate and variance, and percentage of low melatonin). Adjusted means, standard errors, and 95% confidence intervals were derived from least squares regression analyses. Multiple logistic regression was used to assess the relation between flight attendant work and the highest or lowest quartiles of the three melatonin measures (corresponding to the lowest or highest 25% of the study values in the direction of the hypothetical circadian disruption). The relative odds of falling into the highest or lowest quartile and the 95% confidence interval (95% CI) for the odds ratio (OR) were calculated. Adjusted means and odds ratios were not reported when the crude relation best fit the data.

The daily overnight melatonin rates were also evaluated in a linear mixed-effects model with flight attendant or teacher status as a fixed effect and subject as a random effect. Multiple observations collected over time from the same woman are likely to be correlated, and a mixed-effects model allows the covariance structure to be modeled along with the fixed effects. The considered covariance structures included compound symmetry, which assumes that observations from the same woman have homogeneous variance and homogeneous covariance, and autoregressive with a random subject effect, which assumes that observations from the same woman have homogeneous variance but a covariance that decreases exponentially as the interval between observations increases. The selection of a covariance structure relied on patterns observed in the data, Akaike's information criterion, Schwarz's Bayesian information criterion, and the likelihood ratio test (21). Adjusted melatonin rates and between- and within-woman variance estimates were produced for the flight attendants and teachers.

Frequency distributions and stratified analyses were used to characterize the relation between the melatonin measures and flight attendant or teacher status initially and to assess the evidence of confounding and effect modification by demographic, medical, and life-style variables. Previous characterization of the participants indicated differences between the flight attendants and teachers for body mass index (BMI) and several other demographic and life-style variables (14). However, the mean ages of the flight attendants and teachers were similar, 36.0 (SD 4.7, range 27–45) years versus 37.4 (SD 5.9, range 24–45) years, respectively. Logarithmic and arcsine transformations were used to normalize the melatonin variance and the percentage of low melatonin, respectively. The initial models contained BMI as an a priori potential confounder and all other potential confounders with a P-value less than or equal to 0.2. The screening for effect modifiers was limited to age and BMI, on the basis of previous analyses and the literature; no product terms were entered into the initial models or retained in the final models. Regression analyses were conducted with modified backwards selection of the covariates causing 15% or greater change in the exposure coefficient. Residual, multicollinearity, and goodness-of-fit analyses were conducted to confirm that the final models did not violate analytic assumptions.

The following variables were evaluated as potential confounders: (i) demographic information, including age, race, Hispanic origin, education, income; (ii) daily and weekly diary information, including use of sleep, analgesics or nonsteroidal antiinflammatory agent medications, alcohol and caffeine consumption, minutes of exercise outside work, heavy lifting, chance to relax during the week, attempt to diet during the week; (iii) daily urinary cotinine concentrations to reflect active and passive smoking; (iv) medical and physical factors, including BMI, and report of a current cold; (v) work environment, decision latitude (22), job strain (23), acute and chronic stressors, including five or more hours of secondary employment per week, sharing of household duties, presence of a hostile living or work environment, care-giver status, body image, and stress events during the study period and 6 months preceding it.

To group the 16 measures or indicators of circadian rhythm disruption descriptively (table 1), we used correlation analysis, exploratory principal components analysis, and factor analysis. Principal components and factor analyses are methods that can reduce a set of correlated variables to a smaller set of conceptually meaningful variables called components or factors. Initially in the principal components analysis, components were retained to account for at least 75% of the total variability of the original data. Selected components were then rotated with a varimax rotation to aid interpretation. Variables with rotated factor loadings above 0.4 on a factor were considered associated with the factor.

Results

Crude and adjusted values for the three melatonin-based metrics of circadian disruption are presented in table 2. The variance of the overnight melatonin rate was

Table 2. Crude and adjusted means, odds ratios (OR) for the highest or lowest quartile, and 95% confidence intervals (95% CI) for three measures of melatonin production in the 39 flight attendants and the comparison group of 24 teachers.

Outcome	Crude mean	95% CI for the crude mean	Adjusted mean [a]	95% CI for the adjusted mean	Crude OR for highest or lowest quartile	95% CI for the crude OR	Adjusted OR [a] for highest or lowest quartile	95% CI for the adjusted OR
Mean melatonin rate ($\times10^3$ ng/h)								
Flight attendants	1.4	1.2–1.6	·	·	0.20	0.1-0.7 [b]	·	·
Teachers	1.2	1.0-1.5	·	·	1.0	·	·	·
Variance, melatonin rate [$\times10^5$ (ng/h)^2] [c]								
Flight attendants	2.4	1.6-3.4	2.8	1.8-4.6 [d]	2.2	0.6-7.9 [e]	2.3	0.6- 9.1 [e, f]
Teachers	1.3	0.8-2.1	1.0	0.5-1.9 [d]	1.0	·	1.0	·
Percentage of low melatonin days [g]								
Flight attendants	31.2	24.1-38.7	·	·	0.7	0.2-2.3 [h]	·	·
Teachers	31.4	22.5-41.0	·	·	1.0	·	·	·

[a] Not provided where the crude relation best fit the data.
[b] The odds of a flight attendant being in the lowest quartile of the mean melatonin rate relative to that of the teachers. The odds of a teacher being in the lowest quartile of the mean melatonin rate relative to that of the flight attendants was 4.9 (95% CI 1.4-16.8).
[c] Melatonin variance was normalized with log transformation prior to the analysis and then untransformed for the reported results.
[d] Adjusted for education, decision latitude, and use of sleep-inducing medication.
[e] The odds of a flight attendant being in the highest quartile of melatonin rate variance relative to that of the teachers.
[f] Adjusted for income and presence of a hostile living or work environment.
[g] The percentage of the low melatonin days was calculated as the proportion of monitoring days for which the rate was less than 50% of the mean of a woman's highest four rate days, normalized with arcsin transformation prior to the analysis, and then untransformed for the reported results.
[h] The odds of being in the highest quartile of the percentage of low melatonin days relative to that of the teachers.

associated with flight attendant occupation, although some precision was lost in the categorical modeling by quartile. Flight attendants experienced increased circadian rhythm disruption, as measured by a higher adjusted melatonin rate variance, than the teachers (2.8×10^5 versus 1.0×10^5 $(ng/h)^2$, respectively; P=0.04; adjusted for education, decision latitude, and use of sleep-inducing medication) and the likelihood of being in the highest quartile of melatonin variance (OR 2.3, 95% CI 0.6–9.1, adjusted for income and hostile environment). The mean overnight melatonin rates of the flight attendants and teachers were similar. However, the crude odds of a flight attendant's melatonin rate falling in the lowest quartile compared with a similar finding for the teachers was 0.2 (95% CI 0.1–0.7). This finding indicates that the teachers were 4.9 times as likely as the flight attendants to have a melatonin rate in the lowest quartile (95% CI 1.4–16.8). The percentage of days with a low melatonin rate was not associated with occupation.

The results of the associated repeated-measures analysis (table 3) were consistent with the melatonin rate and variance findings from the regression analyses of table 2. A total of 2130 daily overnight melatonin rates obtained for 63 women were used in the linear mixed-effects model. The compound symmetric covariance structure provided the best fit for the data. In addition, fitting separate covariance structures for flight attendants and teachers, as opposed to a common structure, was supported by the likelihood ratio test (χ^2=61.0, 2 degrees of freedom). The mean overnight melatonin rate was similar for the flight attendants and teachers, although the flight attendants' mean rate was slightly higher (1.5×10^3 versus 1.0×10^3 ng/h, respectively; adjusted for BMI, decision latitude, and hostile environment). The flight attendants exhibited more within-woman variability than the teachers [4.3×10^5 versus 2.6×10^5 $(ng/h)^2$, respectively], and the flight attendants exhibited less between-women variability than the teachers [2.7×10^5 versus 5.0×10^5 $(ng/h)^2$, respectively].

Table 4 provides the Spearman correlation coefficients for the 16 measures or indicators of circadian rhythm disruption. The results of the exploratory principal components analysis with varimax rotation are presented in table 5. The first three components accounted for 52.8%, 13.5%, and 9.1% of the total variance, respectively. Each of the three factors was related to melatonin production. Time zones crossed correlated with melatonin production and many measures of sleep displacement.

The first factor can be described as "sleep, work, and time zone displacement". Most of the variables that loaded on this factor describe conditions in which the timing of sleep and work were shifted erratically so that sleep and sample collection often took place outside the woman's standard nocturnal sleep interval and work was often performed during this interval. These variables included negative loadings on nocturnal sleep fraction and home phase proportion and positive loadings on the mean and variance of the midpoint of the main sleep period, average and cumulative time spent working during normal sleep hours and the mean and variance of activity acrophase. Furthermore, the average and cumulative time zones crossed were loaded positively on this factor, a finding suggesting that transmeridian travel correlates with variable shifts in the sleep- and worktimes of flight attendants. Total sleep time in the main sleep period was loaded positively on this factor and, therefore, indicated that greater displacement of sleep and work from typical times correlated with more sleep in the main sleep period of the day.

The second factor can be described as "melatonin and time zone displacement". It included the mean and variance of the overnight melatonin rate, the average and cumulative time zones crossed, and the cumulative time spent working during normal sleep hours. The positive loadings suggest that crossing more time zones correlated with higher and more variable melatonin rates. The third factor, which we describe as "melatonin and sleep

Table 3. Mixed-effects model results for the daily overnight melatonin rate—unadjusted and adjusted models for the 39 flight attendants and 24 teachers. (95% CI = 95% confidence interval)

	Woman-days	Least squares mean		Within-woman variance		Between-women variance	
		10^3 ng/h	95% CI	σ^2_w [10^5 $(ng/h)^2$]	95% CI	σ^2_b [10^5 $(ng/h)^2$]	95% CI
Unadjusted model							
Flight attendants	1267	1.4	1.2-1.6	4.3	4.0-4.6	3.0	1.6-4.4
Teachers	863	1.2	0.9-1.5	2.6	2.4-2.9	4.7	1.9-7.4
Adjusted model [a]							
Flight attendants	1267	1.5	1.2-1.9	4.3	4.0-4.6	2.7	1.4-4.1
Teachers	863	1.0	0.6-1.5	2.6	2.4-2.9	5.0	2.0-8.1

[a] Adjusted for body mass index, decision latitude, and hostile environment.

Table 4. Spearman rank correlation coefficients for 16 measures or indicators of circadian rhythm disruption.[a, b] (MEL = melatonin, Low MEL = percentage of low melatonin, HPP = home phase proportion, Sleep fraction = nocturnal sleep fraction, TST = total sleep time, MSP = main sleep period, mid = midpoint, ACT ACR = activity acrophase, TZ = time zones, cum = cumulative, TZ mean = mean number of time zones crossed/flight, SSI = standard sleep interval, Mean SSI travel = mean SSI travel/flight)

	MEL variance	Low MEL	HPP mean	HPP variance	Sleep efficiency	Sleep fraction	TST in MSP	MSP mid mean	MSP mid variance	ACT ACR mean	ACT ACR variance	TZ cum	TZ mean	SSI travel cum	SSI tavel mean
MEL rate	**0.75**[b]	–0.13	–0.02	–0.03	**–0.25**	–0.05	0.21	–0.20	–0.06	–0.17	–0.10	**0.24**	**0.23**	0.18	0.20
MEL variance		**0.42**	**–0.22**	**0.25**	–0.14	**–0.27**	**0.25**	0.08	0.20	0.06	**0.24**	**0.44**	**0.43**	**0.33**	**0.38**
Low MEL			–0.15	**0.25**	0.03	–0.21	0.06	**0.23**	0.20	0.14	**0.23**	0.17	0.16	–0.03	0.03
HPP mean				**–0.92**	–0.03	**0.78**	**–0.50**	**–0.39**	**–0.65**	**–0.46**	**–0.56**	**–0.50**	**–0.52**	**–0.49**	**–0.48**
HPP variance					0.05	**–0.73**	**0.46**	**0.42**	**0.60**	**0.46**	**0.56**	**0.47**	**0.51**	**0.46**	**0.46**
Sleep efficiency						0.15	**–0.23**	0.20	–0.07	0.10	0.03	–0.08	–0.13	–0.07	–0.07
Sleep fraction							**–0.60**	**–0.27**	**–0.79**	**–0.36**	**–0.69**	**–0.49**	**–0.55**	**–0.46**	**–0.48**
TST in MSP								0.10	**0.44**	**0.29**	**0.27**	**0.47**	**0.54**	**0.51**	**0.54**
MSP mid mean									**0.36**	**0.90**	**0.54**	**0.39**	**0.40**	**0.43**	**0.42**
MSP mid variance										**0.44**	**0.73**	**0.50**	**0.52**	**0.58**	**0.57**
ACT ACR mean											**0.53**	**0.41**	**0.43**	**0.53**	**0.50**
ACT ACR variance												**0.53**	**0.54**	**0.60**	**0.59**
TZ cum													**0.95**	**0.88**	**0.86**
TZ mean														**0.86**	**0.90**
SSI travel cum															**0.96**

[a] The number of observations for each correlation varied from 55 to 65 due to missing data.
[b] Values in bold indicate Spearman rank correlation coefficients with associated P-values of ≤0.10.

efficiency", includes a negative loading for the mean melatonin rate and positive loadings for the percentage low melatonin, sleep efficiency, and the midpoint of the main sleep period. The loadings on these factors suggest that low melatonin correlates with increased sleep efficiency.

Discussion

In our study, flight attendants experienced more circadian disruption, as measured by more variable melatonin rates, than a minimally flying comparison group. We also found that, for studies of flight attendants in which even a simplified field measurement of melatonin is infeasible, time zones crossed, derived from flight history information, may be a useful indicator of both sleep displacement and melatonin desynchronization. For large epidemiologic studies of flight crews, time zones crossed can be used to examine relations between circadian disruption and reproductive health endpoints.

The variance of the flight attendants' overnight melatonin rate was greater than that of the teachers', when modeled with both summary and daily rate data. The analyses are consistent with each other and with the melatonin phase variability that Sack et al observed in more detail for shift workers (11). In their study, shift workers had greater variability than day workers in the timing of melatonin production, as well as an average peak in production earlier in the day than day workers. In addition, in contrast to the day workers, the shift workers' melatonin peak did not generally coincide with their self-selected sleep times. It is difficult to achieve consolidated sleep for 8 hours or more when sleep is attempted at an unfavorable circadian phase (9). A favorable phase of sleep coincides with the time of high melatonin production. The increased variance of flight attendants' overnight melatonin rates suggests that misalignment of the sleep-wake cycle and circadian rhythms may be a chronic occupational condition that may not be readjusted fully by rest after each duty period. Rest requirements for flight attendants (24) may address the recovery of conditions such as alertness, which may readjust more quickly than the recovery time required for the full resynchronization of internal rhythms and exogenous circadian rhythm components (25).

There is evidence that reproductive health may be affected by desynchronization. Recently, studies have indicated an association between increasing years of night-shift work and risk of breast cancer (26, 27). Melatonin may affect, or be affected by, the production of sex steroids, as well as play a role in the intraovarian regulation of steroidogenesis (10). Elevated melatonin levels have been measured for women with secondary amenorrhea, perhaps due to decreased estrogen levels (28). We are currently evaluating the reproductive hormone profiles of the flight attendants and teachers in this study.

Table 5. Factor analysis loadings of 16 measures or indicators of circadian rhythm disruption.[a] [HPP = home phase proportion, MSP = main sleep period (which can occur at any time during each 24-hour period), SSI = standard sleep interval (the time between 2200 and 0800, home base time, "home base time" being the time within the time zone of the domicile in which a flight attendant was based or the city in which a teacher lived)]

Measure or indicator	Factor loading		
	Factor 1 (52.8%)[b] (sleep, work, and time zone displacement)	Factor 2 (13.5%) (melatonin and time zone displacement)	Factor 3 (9.1%) (melatonin and sleep efficiency)
Mean melatonin rate	-0.17	**0.78**	**-0.41**
Variance of melatonin rate	0.03	**0.82**	0.08
Percentage of low melatonin	0.02	0.33	**0.68**
HPP mean	**-0.95**	-0.10	0.002
HPP variance	**0.94**	0.08	-0.09
Sleep efficiency	-0.08	-0.10	**0.59**
Nocturnal sleep fraction	**-0.94**	-0.13	0.02
Total sleep time in MSP	**0.70**	0.11	-0.32
MSP midpoint	**0.76**	-0.03	**0.43**
Variance of the MSP midpoint	**0.95**	0.09	-0.01
Activity acrophase	**0.82**	-0.07	0.33
Activity acrophase variance	**0.95**	0.005	-0.07
Cumulative number of time zones crossed	**0.48**	**0.64**	0.24
Mean number of time zones crossed/flight	**0.49**	**0.62**	0.17
Cumulative SSI travel	**0.80**	**0.42**	0.02
Mean SSI travel/flight	**0.84**	0.32	0.02

[a] Boldface variables were considered part of the factor and loaded at ≥0.4 in a varimax factor rotation of data from 31 flight attendants and 20 teachers.
[b] Percentage of data variance explained by the factor.

The average overnight melatonin rate was slightly higher for the flight attendants than for the teachers, and the teachers' melatonin rates were about five times as likely as the flight attendants to fall into the lowest quartile. We had hypothesized the opposite result, namely, that decreased overnight rates would indicate disruption, since a decreased rate could indicate either suppression or a shifting of the characteristic 24-hour rhythm of melatonin. However, our findings are consistent with those of some previous studies. Shift workers with melatonin amplitudes (difference between maximum and mean values) four times those of referents were reported by Touitou et al (29), and melatonin increases have been reported in at least one other shiftworker study (30) and have also been reported for persons experimentally subjected to sleep deprivation (31, 32). Several explanations have been suggested for this finding; the most plausible may be that the increase in melatonin is an adaptive or compensatory response to shift work or sleep deprivation.

Our factor analysis suggests that time zones crossed reflects circadian rhythm disruption. In a sense, our study findings may represent an underestimate of the magnitude of circadian disruption, since they are based upon a group of flight attendants whose flights likely crossed fewer time zones than flights from domiciles servicing predominantly east-west routes. Flight attendants with extensive transmeridian travel would be likely to score high on the first two factors, which both contain time zones crossed and account for two-thirds of the variance in the data.

The third factor describes the smallest amount of data variance of the three factors and includes positive loadings for sleep efficiency and the proportion of low melatonin days. When the factor analysis was stratified by occupation, the pattern for the flight attendants was consistent with the analysis of combined data reported in table 5. Sleep efficiency was prominent in several of the factors for the small number of teachers (N=20). The questions correlated with sleep efficiency will be further evaluated among a larger group (approximately 2500) of flight attendants and teachers who are taking part in another NIOSH study.

Our factor analysis was descriptive rather than etiologic. Alternate analytic criteria can create other factor structures with differing interpretations, including the separation of variables we observed as correlated. However, to examine the predictive power of time zones in a more quantitative fashion, we also performed an alternate analysis in which the variables for time zones crossed and those for standard sleep interval (SSI) travel were omitted from the factor analysis and factor scores for each person were generated and correlated with the time zone and SSI travel variables. In this alternative analysis, the factor analysis produced three factors that were similar to those reported in table 5, except that the time zone and SSI travel variables were excluded. The first factor contained sleep and activity variables, the second factor contained melatonin mean and variance, and the third factor contained sleep efficiency and the percentage of low melatonin days. Factor scores were computed for each study participant and correlated with the time zone and SSI travel variables. For the flight attendants, the time zone variables were significantly correlated with the scores of both factor 1 (sleep variables) and factor 2 (melatonin variables). The SSI travel variables were only correlated with the factor 1 scores. These alternative analysis results support the relation of time zones crossed to measures of sleep displacement and melatonin desynchronization, which we have reported. We also observed the correlation of time zones crossed with measures of disruption and displacement prior to rotation and in the preliminary correlation analyses, and the patterns observed in the selected three-factor structure were consistent with the results of our previous analyses and the understanding of sleep displacement among flight attendants and teachers (16). Thus the use of time zones crossed to indicate circadian disruption is based upon the results of both our factor analysis and additional information.

The use of time zones crossed to assess circadian disruption has some potential limitations. Flight attendants who fly predominantly long north-south routes (eg, Miami to South America, crossing 0–2 time zones) or short commuter routes may incur sleep displacement, but they travel across fewer time zones than flight attendants who work predominantly on long east-west routes (eg, Seattle to Australia, crossing 18 time zones). Because sleep is a component of circadian disruption, we plan also to incorporate measures of sleep displacement (eg, SSI flight time) into our upcoming reproductive health studies to evaluate sleep displacement among these flight attendants. A flight attendant who starts and ends the day at her home base could cross and recross a minimal number of time zones throughout the day, the result being a slightly higher classification of exposure to circadian disruption than her actual exposure. An examination of the flight attendant work histories of our study suggests that this possibility does not appear to be a major source of misclassification. The presence and magnitude of this potential misclassification will be evaluated from two million work history flight segments in an upcoming NIOSH study.

The correlations in the factor analysis between time zones crossed, sleep displacement, and increased main sleep duration and sleep efficiency were unexpected. Nonetheless, we have previously shown that the flight attendants in our study had reduced sleep efficiency and more wakefulness during the main sleep period than teachers, despite longer total sleep times (16). Taken together, these findings suggest that flight attendants may spend more time in bed attempting to sleep than teachers do, especially after crossing many time zones, perhaps in an effort to compensate for the sleep difficulties induced by their circadian disruption. Actigraphs tend to overestimate sleep when people lie awake but motionless in bed (19). Although we used actigraph event markers and diaries to improve the accuracy of our sleep data, it is possible that the degree of sleep disruption induced by flight attendant's time zone crossings was underestimated.

The interpretation of our results is limited by the small number of women included in this feasibility study. The desynchronization of melatonin was assessed by calculating an overnight melatonin rate from daily first-morning urine samples instead of using less feasible continuous sampling in the field. In addition, we were not able to measure concurrent light exposures with activity in the field when the study was conducted. Light exposure measurement may have been desirable, since melatonin production can be suppressed by light exposure and the light-dark cycle is the most important synchronizer of the human circadian system (33, 34). It is possible that lighting differences experienced by flight attendants and teachers may have contributed to the difference in melatonin levels; however, this is speculative without measurements of actual light exposure. Despite these limitations, we were able to characterize disruption in this working population and identify useful exposure metrics for future epidemiologic studies. A comprehensive analysis of exogenous and endogenous contributors to disruption may not be necessary in similar studies (25).

America is a nation of tired workers. In 1990, the direct costs of sleep disorders and deprivation were estimated at USD 15.9 billion, and the indirect costs resulting in stress-related, reduced workplace productivity were estimated at USD 150 billion (35). Our results are relevant specifically to flight attendants, but they also highlight the need for research on sleep and circadian rhythms to provide useful guidance for working populations.

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