Original article

SJWEH Suppl 2006;(2):27–34

Diurnal cortisol pattern of shift workers on a workday and a day off

by Björn Karlson, PhD,[1] Frida Carlsson Eek, PhD,[1] Åsa Marie Hansen, PhD,[2] Anne Helene Garde, PhD,[2] Kai Österberg, PhD,[1] Palle Ørbæk, MD [2]

Karlson B, Carlsson Eek F, Hansen ÅM, Garde AH, Österberg K, Ørbæk P. Diurnal cortisol pattern of shift workers on a workday and a day off. *SJWEH Suppl* 2006;(2):27–34.

Objectives The aim of this study was to determine how the diurnal rhythm of the hypothalamus-pituitary-adrenal axis is affected by a fast forward-rotating 24-hour shift schedule and to explore possible relationships with self-reported health, sleep-related problems, and recovery-related problems.

Methods Shift workers on their morning (N=45) or afternoon (N=32) shift were compared with daytime workers (N=39) from the same worksite and with an external daytime working reference group with early (N=50) or late (N=130) waking. Cortisol in saliva was sampled at waking, after 30 minutes, after 8 hours, and at 2100 on a daytime workday for all of the groups and also on a day off for the shift workers. Sleep and subjective health complaints were assessed with a questionnaire.

Results The morning shift workers showed a deviant cortisol pattern over the workday, with a lower cortisol level at waking and a lower morning peak level. The morning and afternoon shift workers did not differ with respect to the cortisol level on the day off. The shift workers also reported lower self-rated health and more problems with sleep and recovery.

Conclusions The results suggest that a partial adaptation of the circadian cortisol rhythm to night work does not re-adjust during 4 days off, and hence the early waking on morning shift days occurs during an earlier phase of the diurnal cortisol rhythm than for daytime workers waking up at similar hours. The results may contribute to the understanding of reduced alertness during morning shifts and have implications for the planning of, and adaptation to, shift schedules.

Key terms Karolinska Sleep Questionnaire; saliva; shift work; sleep; Swedish Occupational Fatigue Inventory.

Shift work that includes night work may have various negative physiological and psychosocial effects that can affect health. The effects may depend on such factors as how and to what extent the work schedule interferes with the biological rhythm and the person's recovery ability, social life, and family life (1). The most commonly reported complaints among shift workers with night work are insufficient sleep, sleepiness, and fatigue, and the most clearly shown long-term health effects are gastrointestinal and cardiovascular diseases (2–4). Night work and rotating shift schedules interfere with the normal internal circadian rhythm and sleep–wake cycle. This interference may lead to continuous internal demands to adjust to varying workhours, and sleep disturbances may be a consequence. A partial adaptation to on-going night work occurs, as can be observed through alterations in the diurnal cortisol pattern (5). The release of cortisol normally follows a robust circadian rhythm, with peaking levels in the morning after waking, followed by decreasing levels throughout the day. However, after a number of consecutive nights of work this rhythm has been shown to change in the direction of increased levels and flattened profiles of cortisol during night shifts (6) and result in successively decreasing morning levels (7), approaching lower cortisol concentrations in the morning than in the evening (8).

Most previous studies of endocrine markers have been made in relation to shift and night work, and less is known about the effects of early-morning shifts. From the perspective of risk for long-term health effects, it is, however, the most interesting to focus on recovery and the restitution of physiological effects and wear and tear from the repeated desynchronizations of physiological systems. The aim of our study was to determine how the diurnal rhythm in the hypothalamus-pituitary-adrenal (HPA) axis, as measured by salivary cortisol on a

1 Lund University Hospital, Division of Occupational and Environmental Medicine, Lund, Sweden.
2 National Institute of Occupational Health, Copenhagen, Denmark.

Reprint requests to: Björn Karlson, Division of Occupational and Environmental Medicine, Lund University Hospital, SE-221 85 Lund, Sweden. [Email: bjorn.karlson@med.lu.se]

daytime morning or an afternoon shift on a day off work, is affected by a fast forward-rotating 24-hour shiftwork schedule and to explore possible relationships with self-reported health, sleep-related problems, and recovery-related problems.

Study population and methods

Study population

The study was carried out at a manufacturing plant with blue-collar employees working either a 24-hour shift or a daytime shift. The 24-hour shift schedule was fast forward-rotating in the cycles M M A A N N - - - - (M=morning, A=afternoon, N=night, - =day off), with shift changes at 0600, 1400, and 2200. Daytime workers worked either 0700–1545 or 0800–1630, or on a weekly changing schedule of 0600–1400 and 1400–2200. Of the 369 available employees invited to participate, 283 (77%) responded to a questionnaire, and 117 (32%) also collected salivary samples. Of these, 78 were in blue-collar 24-hour shift work [59 men and 19 women, mean age 44.0 (SD 10.7) years], and 39 were in blue-collar or white-collar daytime work [31 men and 8 women, mean age 47.7 (SD 9.2) years]. One of these workers, who sampled cortisol on his afternoon shift, provided only one analyzable saliva sample and was thus excluded from the analysis of the cortisol data although not from that of questionnaire data.

In addition, data previously collected from white-collar daytime workers from several other worksites of various branches, examined in a similar way, were used as external reference data for the cross-sectional analyses (N=355). This external reference group consisted of 117 men and 238 women [mean age 46.9 (SD 11.1) years] who were slightly older than the shift workers.

Table 1. Waking time and sleep length for the groups during their workday, and for the shift workers also sampling saliva on their day off (N=64). (h:min = hours:minutes)

	Waking time		Sleep length	
	Mean	SD (h:min)	Mean (h:min)	SD (h:min)
All groups on the workday				
Morning shift	0442	0:19	5:47	1:10
Early-waking referents	0448	0:25	5:48	0:50
Afternoon shift	0754	1:09	6:47	1:17
Late-waking referents	0651	0:36	7:07	1:02
Daytime	0611	1:08	6:22	1:02
Saliva sampling shift workers on the workday and day off				
Morning shift workday	0439	0:20	5:43	1:13
Morning shift day off	0822	1:21	7:27	1:26
Afternoon shift workday	0749	1:11	6:51	1:22
Afternoon shift day off	0747	1:35	7:26	1:30

An exploratory analysis of morning cortisol in the total group of shift workers revealed that an earlier awakening was related to a lower cortisol concentration both at waking ($r=0.48$, $P<0.001$) and at the second sampling about 30 minutes later ($r=0.28$, $P=0.02$), but no significant relationship was found for the daytime workers or for the total external reference group. Thus waking time seemed to have an effect only among the shift workers.

Since the shift worker group was comprised of workers on their morning shift, as well as those on their afternoon shift, who woke up at different times (morning shift before 0515 and afternoon shift after 0620), the analyses of the cortisol data were carried out for groups based on waking time, namely, morning shift workers (N=45), afternoon shift workers (N=32), referents (N=50) waking before 0515 (like the morning shift workers), and referents (N=130) waking after 0620 (like the afternoon shift workers). Descriptive data on waking times and sleep length for the groups are given in table 1.

Data sampling methods

Cortisol. The shift workers sampled saliva for the analysis of cortisol concentrations on the second day of a morning (N=45) or afternoon (N=31) shift, and on the third day off work. The samples taken during the day off were used as individual reference values, since they would not be affected by any work-related stress (9, 10) or other aspects associated with the workday. Only 64 of the shift workers sampled saliva on their day off. The daytime workers and the external referents took samples only during one of the middle 3 days of a 5-day workweek. Saliva was sampled five times per day: at waking, 30 minutes after waking, at 1100, 8 hours after waking, and at about 2100, except for the external reference group, who did not take the 1100 sample.

The variables analyzed were (i) the overall cortisol concentration (nmol/l) across the points of sampling over the day, (ii) the awakening response, defined as either the relative increase (the percentage increase) or the absolute increase (nmol/l) in the cortisol concentration from the waking sample to the second sample 30 minutes later, (iii) the maximum morning concentration (either of the first two samples; nmol/l), and (iv) the decline over the day, defined as the difference (in nanomoles per liter) between the maximum morning concentration and the final sample at 2100.

Inventories. The Karolinska Sleep Questionnaire (KSQ) was used to assess sleep-related symptoms and problems during the past 6 months (11). The questionnaire has 15 items rated on a 5-point scale with the response alternatives "never", "rarely (a few times)", "sometimes (some/a few times a month)", "most of the time (some/

a few times a month)", and "always (every day, more or less)". From 12 of the 15 items, the following three indices were calculated: "waking problems" (items: difficulty waking up, not feeling rested on awakening, and exhaustion on awakening), "daytime sleepiness" (items: sleepiness during work or leisure time, irritated or tired eyes, involuntarily falling asleep at work, involuntarily falling asleep during leisure time, and struggling to stay awake), and "sleep disturbances" (items: difficulty in falling asleep, repeatedly waking up and finding it hard to get back to sleep, premature awakening, and disturbed sleep). The external reference group did not fill out the questionnaire, however.

The Swedish Occupational Fatigue Inventory (SOFI-20) was used to assess five dimensions of fatigue after a typical workday (lack of energy, physical exertion, physical discomfort, lack of motivation, and sleepiness), together with a global scale computed as the person's mean score across all items (12). The SOFI-20 contains 20 items, four items for each of the five subscales, and all items are rated on a 7-point scale with two verbal anchors, (i) "not at all" and (vii) "to a very high degree".

Sleep-related and recovery-related problems were also assessed through four single-item questions, rated on 5-point scales with varying labeling. The questions concerned how often the participant felt "fatigue after work" (always to never), the number of days needed for "recovery after a workweek" (<1, 1, 2–3 days, longer, never feel recovered), the degree of satisfaction with "achieved amount of sleep" (definitely enough to far from enough), and the "quality of sleep" (very good to very poor). A similar single-item question also asked the participants to rate their "satisfaction with workhours" on a 5-point scale (very low to very high).

Self-rated health (SRH-7) was used to assess the individual's global experience of his or her health status by a single item question asking how he or she felt right now, physically and psychologically, with respect to health and well-being (13). The question was responded to on a 7-point scale, with the following verbal labels at the end points: (i) "very bad, couldn't feel worse" and (vii) "very good, couldn't feel better".

Procedure

The questionnaires were filled out at the worksite during a daytime shift under the supervision of the research group. On the same occasion, each participant was shown the proper procedure for taking saliva samples, including the instruction to place the swab from the sampling tube in the mouth until hydrated, but no longer than 5 minutes. Each person also received written information together with the set of sampling tubes (Salivette®, Sarstedt Ltd, Leicester, UK). Restrictions were issued concerning teeth-brushing, smoking, and heavy meals 1 hour prior to the saliva sampling. On the day following the saliva sampling, samples were stored at –20°C until analyzed.

Analysis of cortisol in saliva

The assay used for determining cortisol levels in saliva was a competitive radioimmunoassay (RIA) (Spectria Cortisol Coated Tube RIA) purchased from Orion Diagnostica, Espoo, Finland. The assay was designed for quantitative in vitro measurement of cortisol in serum, plasma, urine, and saliva. A method evaluation of certified reference material in water showed no bias of the method, with a 97% recovery [95% confidence interval (95% CI) 94–101]. The limit of detection (LOD) was 1.59 nmol/l. The between-run coefficient of variation (CV) was 19% at 11.5 nmol/l and 16% at 49.2 nmol/l. To show the equivalence between different runs, we used natural saliva samples at two concentrations (11.5 nmol/l and 49.2 nmol/l) as control material and analyzed them together with the samples. Westgard control charts were used to monitor and control variation and ensure that the analytical methods remained stable. The performance of the methods was further evaluated by participation in interlaboratory comparison schemes (14–16).

Data management and statistical analysis

Because of the skewed data distribution, the cortisol concentrations were logarithmically transformed before the statistical analyses were carried out. The variables for awakening response and decline over the day included negative values, and therefore the data were instead ranked before the statistical analysis.

The linear mixed models module in SPSS 13 (SPSS Inc, Chicago, IL, USA, 2001) was used to specify a repeated-measures model for the analysis of cortisol. The models were solved using the restricted maximum likelihood (REML) method. The categorical predictors were group and point of sampling over the day (POS). For the shiftwork group, an analysis of the difference between the workday and day off was also performed, including day as an additional categorical predictor. The statistical modeling also included the two-way interaction group × POS to find possible differences between the groups in patterns of cortisol during the day. For all of the analyses, a series of first-order autoregressive covariance structures was tested, as well as a compound symmetry covariance structure. The Schwarz Bayesian information criterion was used to guide the final selection of the covariance structure. If statistical significance was reached in the final model, posthoc testing was performed. P-values of ≤0.05 were considered statistically significant. The relationships between the cortisol variables and self-report measures were calculated as

Table 2. Cortisol concentrations (nmol/l) four times during a workday.

Sampling time	Shift workers						External referents						Daytime workers (N=39)		
	Morning shift (N=45)			Afternoon shift [a] (N=32)			Early-waking (N=50)			Late-waking (N=130)					
	Median	1st quartile	3rd quartile	Median	1st quartile	3rd quartile	Median	1st quartile	3rd quartile	Median	1st quartile	3rd quartile	Median	1st quartile	3rd quartile
At waking	6.2	4.1	8.3	13.3	9.7	19.3	13.0	9.8	18.9	14.1	9.8	19.8	14.4	11.9	19.8
30 minutes after waking	12.5	10.5	21.0	17.5	14.0	27.2	21.8	17.6	29.4	21.0	14.5	28.6	17.5	14.3	26.9
8 hours after waking	6.1	4.0	10.0	5.2	3.1	7.1	8.3	5.9	11.8	6.5	4.1	10.7	5.2	3.2	6.9
At 2100	4.0	3.0	8.8	2.7	2.2	5.5	3.1	1.8	5.5	3.5	2.1	6.2	2.7	2.3	4.9

[a] One person from the afternoon shiftwork group was excluded from all of the statistical cortisol analyses due to the cortisol samples not being analyzable.

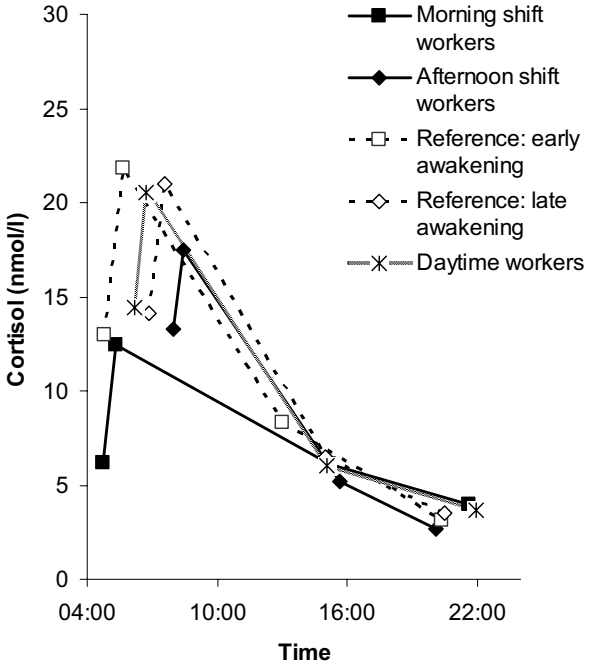

Figure 1. Mean saliva cortisol concentrations at four sampling times (mean time per group) during a workday among morning and afternoon shift workers, early and late awakening external referents, and daytime workers.

partial correlations, with control for waking time and age. The group differences in the self-report measures, as well as for the unrepeated cortisol variables (the daytime workers' awakening response, maximum morning concentration, and decrease over the day) were calculated with general linear modeling in a univariate analysis of variance. In posthoc tests between the shift workers and the other groups, the least square differences of the estimated marginal means were used. Waking time (ie, time at the first sample), self-reported length of sleep during the night preceding the salivary sampling, age, and gender were introduced into the models as covariates for the cortisol analyses, and age and gender were used in the analyses of the questionnaire data if they fulfilled the inclusion criteria of reaching a P-value of <0.2 when introduced into the model (17–19). The group differences in the single-item questions were analyzed with the Kruskal-Wallis test. An analysis of morning cortisol concentration in relation to waking time was carried out within each group separately; for this purpose Pearson's correlations were used.

Ethics

The study was approved by the Ethics Committee of the Medical Faculty, Lund University.

Results

Cortisol on a workday

The descriptive data on cortisol are given in table 2 and figure 1. A model including the two shiftwork groups and the early- and late-waking external reference groups showed a group difference in the overall cortisol concentration during the day (P=0.001). Posthoc tests showed that the morning shift workers had a significantly lower mean cortisol concentration across the workday than the referents waking late (P<0.001) and, more interestingly, also than the referents waking early (P=0.004). Although the afternoon shift workers showed a tendency towards a lower mean cortisol concentration across the day than the late-waking referents did (P=0.09), and they also had a higher mean concentration than the morning shift workers (P=0.06), they did not differ significantly from the other groups (P=0.42 versus early-waking referents). There were no significant differences in the mean cortisol concentration between the referents waking early or late (P=0.43). A model that also included the daytime workers showed similar overall group differences (P<0.001). The posthoc tests showed that the daytime workers, regardless of their waking time, had significantly higher cortisol levels than the morning shiftwork group (P<0.001), but they did not differ from the afternoon shiftwork group

Table 3. Cortisol concentrations (nmol/l) of the shift workers five times during a workday and day off.

Sampling time	Workday						Day off					
	Morning shift workers (N=33)			Afternoon shift workers (N=31)			Morning shift workers (N=33)			Afternoon shift workers (N=31)		
	Median	1st quartile	3rd quartile	Median	1st quartile	3rd quartile	Median	1st quartile	3rd quartile	Median	1st quartile	3rd quartile
At waking	6.4	4.1	9.1	13.1	9.6	19.0	10.9	7.6	17.6	10.2	8.4	18.6
30 minutes after waking	15.1	11.1	21.3	18.3	14.5	30.1	17.8	12.3	25.8	19.6	9.5	26.5
At 1100	5.2	3.9	8.0	8.5	7.4	11.0	10.9	7.2	14.4	8.3	6.3	15.0
8 hours after waking	5.9	4.3	8.5	5.4	3.2	8.0	5.2	3.4	8.5	5.0	3.9	8.6
At 2100	4.1	3.2	9.0	2.9	2.3	6.0	4.9	2.7	7.1	4.8	2.7	8.8

(P=0.11) or from either the early- or late-waking external reference groups in their mean cortisol concentrations across the day (P=0.51 and P=1.0, respectively).

In the model of the four groups there was also a significant interaction effect for group × POS (P<0.001) that indicated differing cortisol patterns over the day. This finding was further analyzed with computed variables and showed a significant group difference with respect to relative awakening response (P=0.004). The posthoc analysis showed that the morning shiftwork group had a higher relative awakening response than the referents with early (P=0.006) and late (P=0.002) waking and the daytime workers (P=<0.001), but its relative awakening response was not higher than that of the afternoon shiftwork workers (P=0.09). However, there were no differences between any of the groups regarding the absolute awakening response (P=0.84).

A significant group difference was also found for the decline over the day (P<0.001). The posthoc analysis showed the decline to be lower in the morning shift group than in all of the other groups (P<0.001), which, in turn, showed a similar decline over the day. A group difference was also found for the maximum morning concentration (P<0.001), for which, again, the morning shiftwork group had significantly lower levels than all of the other groups (P<0.001); no other group differences were observed.

Adjustment for age, gender, waking time, and length of sleep during the night preceding the examination day (table 1) did not substantially change any of the results.

Cortisol on a day off

The descriptive data on cortisol are given in table 3 and figure 2. With adjustment for sleep length, there was an overall difference in the cortisol concentrations over the day between the workday and the day off (P=0.04); this finding indicated higher cortisol concentrations on the day off. However, there was no significant interaction between group and day.

For the computed variables relative awakening response and absolute awakening response, there were no overall effects. For the decline over the day there was a group × day interaction (P=0.002) in which the morning shift workers showed a lower decline on their workday than on their day off (P=0.001) and a lower workday decline when compared with that of the afternoon workers on their workday (P=0.001), as well as on their day off (P=0.03). For the computed variables, the potential covariates did not fulfill the criteria to be included. There was also a similar group × day interaction effect for the maximum morning cortisol concentration (P=0.005) in which the morning shift workers had lower values on their workday than on their day off (P=0.002) and lower workday values than the afternoon workers on their workday (P=0.01), but not on their day off (P=0.07). For both of the variables, the inclusion of

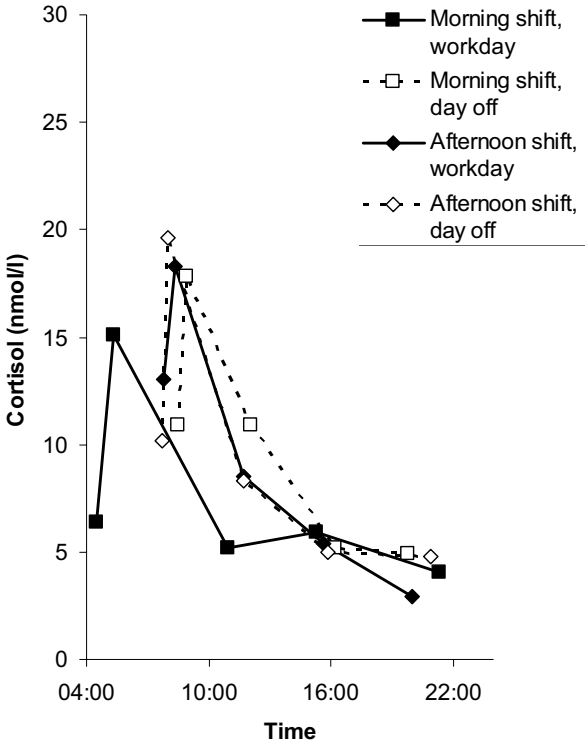

Figure 2. Mean saliva cortisol concentrations at five sampling times (mean time per group) during a workday and during a day off among the morning and afternoon shift workers (N=64).

waking time made the interaction effect disappear even when it did not fulfill the formal inclusion criteria.

Inventories

There was, as expected, no difference in any of the self-report scales between the shift workers who worked the morning or afternoon shift during the day of the saliva sampling. For this reason the results are not shown separately in the tables for these subgroups.

The shift workers reported more physical exertion and discomfort, more lack of motivation, and a higher degree of sleepiness than either of the other groups, but the groups did not differ concerning lack of energy (SOFI-20) (table 4). Furthermore, the shift workers had poorer self-rated health than the other two groups (SRH-7) and reported more frequent waking problems, daytime sleepiness, and sleep disturbances (KSQ) than the daytime workers did. Congruently, the single-item questions regarding sleep- and recovery-related problems showed more frequent feelings of fatigue after work, poorer quality of sleep, a less-satisfactory amount of sleep, and a need for a longer recovery time after a work week among the shift workers than among the daytime workers. The shift workers also reported less satisfaction with their workhours (table 5).

Discussion

The main findings or our study were that the shift workers on a morning shift day showed a clearly deviating diurnal cortisol pattern when compared with the daytime workers awakening either as early or as late as the shift workers, but not compared with the shift workers on an afternoon shift. The morning shift group had lower salivary cortisol concentrations at waking, followed by a higher relative, although not absolute, awakening response. The morning shift group did not reach a morning peak level as high as that of the daytime workers,

Table 4. Inventory results for the groups of shift and daytime workers and for an external reference group of daytime workers from various branches and worksites.[a] (ANOVA = analysis of variance, SOFI-20 = Swedish Occupational Fatigue Inventory, SRH-7 = self-rated health)

Inventory	Shift workers (N=78)			Daytime workers (N=39)			External referents (N=355)			P- value for ANOVA group	P- value for shift workers versus daytime workers	P- value for shift workers versus external referents
	Median	1st quartile	3rd quartile	Median	1st quartile	3rd quartile	Median	1st quartile	3rd quartile			
Karolinska Sleep Questionnaire												
Waking problems	3.0	2.0	3.5	2.3	2.0	3.0	..	..	..	0.003	..	..
Sleep disturbances	3.0	2.3	3.8	2.5	2.0	3.3	..	..	..	0.005	..	..
Daytime sleepiness	2.4	2.0	3.0	2.2	1.6	2.4	..	..	..	0.004	..	..
SOFI-20												
Lack of energy	2.0	0.2	3.3	1.8	0.5	2.8	2.3	1.0	3.8	0.35	..	..
Physical exertion	0.8	0	1.5	0.3	0	1.0	0.3	0	0.8	<0.001	0.03	<0.001
Physical discomfort	2.0	0.5	3.5	1.3	0.3	2.5	1.0	0.3	2.0	<0.001	0.03	<0.001
Lack of motivation	1.3	0.4	2.3	0.5	0.3	1.3	0.8	0	1.8	0.007	0.03	0.002
Sleepiness	2.3	0.8	3.8	1.7	0.5	2.3	1.5	0.7	3.0	0.004	0.008	0.002
SRH-7	4	3	5	5	4	5	5	4	5	0.003	0.045	0.001

[a] Age and gender were included in the ANOVA when appropriate (ie, when P<0.20), and the P-values have been adjusted accordingly. For the SOFI-20 variables, ranked data were used in the ANOVA due to skewed distributions.

Table 5. Group differences for single items regarding sleep- and recovery-related problems and satisfaction with workhours.

Variable	Shift workers (N=78)			Daytime workers (N=39)			External referents (N=355)			Group (P-value[a])	Shift workers versus daytime workers (P-value[a])	Shift workers versus external referents (P-value[a])
	Median	1st quartile	3rd quartile	Median	1st quartile	3rd quartile	Median	1st quartile	3rd quartile			
Fatigue after work	3.0	2.0	3.0	3.0	3.0	3.0	3.0	2.0	3.0	0.04	0.04	0.02
Poor sleep quality	3.0	2.0	4.0	3.0	2.0	3.0	2.0	2.0	3.0	<0.001	0.01	<0.001
Insufficient sleep	3.0	2.0	4.0	2.0	2.0	3.0	2.0	2.0	3.0	<0.001	0.003	<0.001
Days for recovery	3.0	2.0	3.0	2.0	1.0	2.0	2.0	1.0	3.0	<0.001	<0.001	<0.001
Satisfaction with workhours	3.0	2.0	4.0	4.0	4.0	5.0	4.0	4.0	5.0	<0.001	<0.001	<0.001

[a] P-values computed with the Kruskall-Wallis test.

and therefore the cortisol curve flattened out over the day. After 3 days off, the shift workers on a morning and an afternoon shift showed similar and normal cortisol levels and patterns during the day and evening.

Some authors have shown that a lower waking cortisol concentration is related to an earlier waking time (9, 20), while others have not found such a relation or, instead, found an inverse relation (10, 21–23). In our present study we found no relationship between waking time and the concurrent cortisol concentrations at all among the daytime workers, either as correlations within the whole groups or as differences between early- and late-waking reference workers defined in accordance with the waking times of the two shiftwork groups. Low morning cortisol concentrations, as found among the morning shift workers in the present study, have not been found in other data from our research group on people starting work equally early as the morning shift group, but working only daytime up to 12 hours per day (Hansen, personal communication).

Thus, within this study, as well as between several other studies, the findings of relationships between awakening time and cortisol secretion are contradictory or differential. This circumstance may indicate that waking time does not function as a determinant of waking cortisol concentrations per se but rather in relation to the person's internal circadian cortisol and sleep-wake rhythm.

We do not have more-detailed data on the sleep characteristics or other biological indicators of circadian rhythm in conjunction with the present salivary sampling. However, a plausible reason for the low waking cortisol concentration of the morning shift workers may be that they had an altered circadian rhythm with a forward-adjusted phase shift. Thus, at waking, they may have been in an earlier phase of their diurnal cortisol rhythm than the early-waking participants in the reference groups, an effect similar to that described for ongoing night work (8). This possibility could be due to the preceding two night shifts causing a partial adjustment of the diurnal cortisol rhythm with a phase shift forward, as previously shown (6, 8, 24). The sleep phase was subsequently not completely readjusted during the following 4 days off work and 1 day in morning shift work. We do not know the reasons for the early waking hour observed among a minority of the external referents [N=50 of 355 (14%)], but, since waking time was not related to their waking cortisol concentrations, it seems reasonable to assume that they woke up in accordance with a stable internal circadian rhythm, possibly due to being "morning types" or having stabilized early waking times for reasons unknown to us.

Following their low waking cortisol concentrations, the morning shift workers had higher relative cortisol awakening responses, although not higher absolute cortisol increases. Neither did they reach a morning peak level as high as that found in the other groups on the workdays. The reason for the lower peak level could possibly be that the cortisol peak was not captured due to a prolonged increase in cortisol secretion that continued beyond the second measurement of cortisol 30 minutes after waking. Such findings of a prolonged cortisol increase have been reported for early-waking persons in contrast to late-waking persons (22, 25).

Axelsson et al (7) showed morning cortisol to be reduced during an entire extremely rapidly rotating shift cycle of seven work periods over 27 days followed by a week off. They interpreted this finding as reflecting a down-regulated HPA axis as an adaptation to long-term stress. The shift workers on the afternoon shift tended to show an intermediate overall level of cortisol across the day in the group comparisons, and this result cannot be ruled out as indicating a trend towards lowered HPA-axis activation. There were indeed other clear signs of strain among the shift workers, such as poorer self-rated health and more-frequent sleep- and recovery-related problems. However, the very low cortisol levels of the morning shift workers on their workday seem, in the first place, to have been temporary and related to the morning shift rather than to a more permanent down-regulation of HPA-axis activity, since their cortisol concentrations on their day off were similar to those of the afternoon shift workers.

No difference in the cortisol concentrations between the workday and the day off was found for the afternoon shiftwork group. Higher cortisol levels on the workdays than on the days off have often been reported among daytime workers and have commonly been interpreted as an effect of anticipated stress on the workday (10). Possible alternative interpretations of such differences may be a later waking time or a less distinct decision about when waking occurs during days off (9). The lack of such a difference among our afternoon shift workers may be a result of their waking up at the same time on their workday and their day off, as a consequence of the suggested phase shift forward, as already discussed.

If we are correct in the interpretation of our findings, in that a low morning cortisol level on the morning shift day does reflect waking in an earlier circadian phase, they contribute to the understanding of both the affected degree of alertness and sleepiness and the experienced hardship associated with morning shifts, as previously shown (26, 27). They may also have practical implications for decisions about the design of shift schedules and the choice of starting time for the morning shift, as well as for adaptation strategies for shift workers.

A methodological point suggested by the results is that waking time may not predict morning cortisol con-

centrations per se, but rather in relation to the phase of the person's internal circadian rhythm in which waking occurs. For a better understanding of the restitution and circadian adjustment process in relation to night work, a more extensive monitoring of sleep, alertness, and endocrine changes throughout an entire shift cycle, including days off, should be carried out.

In conclusion, we found that, when a person works on a fast forward-rotating shift schedule, the diurnal profile of cortisol is altered during the early morning shift, but not during the afternoon shift or during days off.

Acknowledgments

This study was supported financially by the Swedish Work Environment Authority.

Roger Persson, Lisbeth Prahl, Birgitta Pålsson, and Gunnel Åbjörnsson collected data, and Lisbeth Prahl scanned the questionnaires.

References

1. Costa G. Shift work and occupational medicine: an overview. Occup Med (Lond). 2003;53(2):83–8.
2. Bjorvatn B, Kecklund G, Akerstedt T. Rapid adaptation to night work at an oil platform, but slow readaptation after returning home. J Occup Environ Med. 1998;40(7):601–8.
3. Akerstedt T, Fredlund P, Gillberg M, Jansson B. Work load and work hours in relation to disturbed sleep and fatigue in a large representative sample. J Psychosom Res. 2002;53(1): 585–8.
4. Knutsson A. Health disorders of shift workers. Occup Med (Lond). 2003;53(2):103–8.
5. Weibel L, Spiegel K, Follenius M, Ehrhart J, Brandenberger G. Internal dissociation of the circadian markers of the cortisol rhythm in night workers. Am J Physiol. 1996;270(4 Pt 1):E608–13.
6. Lac G, Chamoux A. Biological and psychological responses to two rapid shiftwork schedules. Ergonomics. 2004;47(12): 1339–49.
7. Axelsson J, Akerstedt T, Kecklund G, Lindqvist A, Attefors R. Hormonal changes in satisfied and dissatisfied shift workers across a shift cycle. J Appl Physiol. 2003;95(5):2099–105.
8. Hennig J, Kieferdorf P, Moritz C, Huwe S, Netter P. Changes in cortisol secretion during shiftwork: implications for tolerance to shiftwork? Ergonomics. 1998;41(5):610–21.
9. Williams E, Magid K, Steptoe A. The impact of time of waking and concurrent subjective stress on the cortisol response to awakening. Psychoneuroendocrinology. 2005;30(2): 139–48.
10. Kunz-Ebrecht SR, Kirschbaum C, Marmot M, Steptoe A. Differences in cortisol awakening response on work days and weekends in women and men from the Whitehall II cohort. Psychoneuroendocrinology. 2004;29(4):516–28.
11. Kecklund G, Akerstedt T. The psychometric properties of the Karolinska Sleep Questionnaire. J Sleep Res. 1992;1 Suppl 1:113.
12. Ahsberg E. Dimensions of fatigue in different working populations. Scand J Psychol. 2000;41(3):231–41.
13. Eriksson I, Unden AL, Elofsson S. Self-rated health. Comparisons between three different measures: results from a population study. Int J Epidemiol. 2001;30(2):326–33.
14. Hansen ÅM, Garde AH, Christensen JM, Eller N, Netterstrøm B. Validation of a radio-immunoassay and establishment of a reference interval for salivary cortisol in healthy subjects in Denmark. Scand J Clin Lab Invest. 2003;63:1–9.
15. Garde AH, Hansen ÅM, Nikolajsen TB. An inter-laboratory comparison for determination of cortisol in saliva. Accreddit Qual Assur. 2003;8(16):20.
16. Westgard JO, Barry PL, Hunt MR, Groth T. A multi-rule Shewhart chart for quality control in clinical chemistry. Clin Chem. 1981;27(3):493–501.
17. Greenland S. Modeling and variable selection in epidemiologic analysis. Am J Public Health. 1989;79(3):340–9.
18. Maldonado G, Greenland S. Simulation study of confounder-selection strategies. Am J Epidemiol. 1993;138(11):923–36.
19. Mickey RM, Greenland S. The impact of confounder selection criteria on effect estimation. Am J Epidemiol. 1989;129(1):125–37.
20. Van Cauter E, Leproult R, Kupfer DJ. Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. J Clin Endocrinol Metab. 1996;81(7):2468–73.
21. Edwards S, Clow A, Evans P, Hucklebridge F. Exploration of the awakening cortisol response in relation to diurnal cortisol secretory activity. Life Sci. 2001;68(18):2093–103.
22. Kudielka BM, Kirschbaum C. Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. Psychoneuroendocrinology. 2003;28(1):35–47.
23. Pruessner JC, Wolf OT, Hellhammer DH, Buske-Kirschbaum A, von Auer K, Jobst S, et al. Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. Life Sci. 1997;61(26):2539–49.
24. Goichot B, Weibel L, Chapotot F, Gronfier C, Piquard F, Brandenberger G. Effect of the shift of the sleep-wake cycle on three robust endocrine markers of the circadian clock. Am J Physiol. 1998;275(2 pt 1):E243–8.
25. Federenko I, Wust S, Hellhammer DH, Dechoux R, Kumsta R, Kirschbaum C. Free cortisol awakening responses are influenced by awakening time. Psychoneuroendocrinology. 2004;29(2):174–84.
26. Ingre M, Kecklund G, Akerstedt T, Kecklund L. Variation in sleepiness during early morning shifts: a mixed model approach to an experimental field study of train drivers. Chronobiol Int. 2004;21(6):973–90.
27. Kecklund G, Akerstedt T, Lowden A. Morning work: effects of early rising on sleep and alertness. Sleep. 1997;20(3):215–23.