

**Diez, Joaquín J. ; Vigo, Daniel E. ; Pérez Lloret, Santiago ;
Rigters, Stephanie ; Role, Noelia ; Cardinali, Daniel P. ; Pérez
Chada, Daniel**

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Sleep habits, alertness, cortisol levels and cardiac autonomic activity in short distance bus drivers: differences between morning and afternoon shifts

Joaquín J. Diez (1), Daniel E. Vigo (2-4), Santiago Pérez Lloret (2,3), Stephanie Rigters (5), Noelia Role (6), Daniel P. Cardinali (3,4), Daniel Pérez Chada (7)

(1) Departamento de Fisiología, Universidad Austral, Buenos Aires, Argentina; (2) Departamento de Fisiología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina; (3) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina; (4) Departamento de Docencia e Investigación, Facultad de Medicina, Universidad Católica Argentina, Buenos Aires, Argentina; (5) Faculteit der Geneeskunde, Universiteit van Amsterdam, Amsterdam, The Netherlands; (6) Laboratorio Hidalgo, Buenos Aires, Argentina; (7) Departamento de Neumonología, Hospital Universitario Austral, Buenos Aires, Argentina.

Contact information

Daniel Pérez Chada

Departamento de Neumonología, Hospital Universitario Austral

Av. Juan D. Perón 1500,

1635 – Derqui - Pilar

Argentina

Phone: 54-11-4962-1292, Fax: 54-11-4962-1292

E-Mail: dperezchada@gmail.com

Key Words

Shift work, sleep, alertness, autonomic nervous system, salivary cortisol, bus drivers.

ABSTRACT

Objective: To evaluate sleep, alertness, salivary cortisol levels and autonomic activity in the afternoon and morning shifts of a sample of short distance bus drivers.

Methods: A sample of 47 bus drivers was evaluated. Data regarding subjects and working characteristics, alertness (psychomotor vigilance task), sleep habits (Pittsburgh Sleep Quality Index, Epworth Sleepiness Scale, Actigraphy), endocrine stress response (salivary cortisol) and autonomic activity (heart rate variability) were collected.

Results: Sleep restriction was highly prevalent. Drivers in the morning shift slept one hour less than those in the afternoon shift, showed lower reaction time performance, a flattening of cortisol morning-evening difference, and higher overweight prevalence.

Conclusions: The differences found between morning and afternoon shifts point out to the need of the implementation of educational strategies to compensate the sleep loss associated with an early work schedule.

INTRODUCTION

Drowsiness and fatigue have been shown to affect alertness, psychomotor performance and judgment, thereby decreasing the individual's ability to operate vehicles safely and increasing the risk of human error leading to injuries and fatalities (1). There is evidence indicating that sleep restriction leads to a distortion of multiple physiological and cognitive parameters (2). In general, a person's tendency to fall asleep during normal waking hours is increased and psychomotor performance declines after successive days of restricted sleep (3). Daytime sleepiness in professional drivers promotes impaired attention while driving, increasing the risk of traffic accidents, a fact particularly relevant in the public transportation system.

Driving a motor vehicle is a complex task that involves numerous cognitive, perceptual, motor, and decision-making skills (4). Driving needs sustained attention in complex dynamic tasks and detection of changes in the environment to search for potential hazards (5). Working over time is a major concern for many bus drivers. The demand for commuter services varies over the day and over the days during the week and many drivers work in complicated and erratic shift systems (6).

Short distance bus drivers are exposed to a complex interaction with other vehicles, traffic lights, tight schedules, passenger's demands, and the complexity of ground transportation in large urban conglomerates. This needs a high level of alertness to accomplish their task in an efficient and safe manner (7).

Falling asleep while driving accounts for a considerable proportion of vehicle accidents particularly during long distance driving conditions (7). In addition, fatigue is present immediately prior to driver involvement in at-fault critical incidents in short distance bus drivers (8). Although performance seems to be hampered mostly by a night shift and the least by an afternoon shift (9), differences between afternoon and morning shift are also a matter of concern (10). The aim of the present study was to evaluate the working characteristics, sleep habits, alertness, cortisol response and autonomic activity in a sample of short distance bus drivers in the city of Buenos Aires.

METHODS

Subjects

The study group included 47 consecutively, not randomly sampled bus drivers, all males, working in 22 short distance (less than 80 km) bus lines from the Buenos Aires Metropolitan area. The inclusion criterion was the possession of a valid professional driving license. As part of the requirements for obtaining the license, drivers should pass a complete psycho-physical examination every two years (for those drivers aged under 45) or every year (for those drivers aged over 45). The exclusion criterion was the refusal to sign the informed consent. The bus lines were selected according to geographical criteria in order to assure that the subjects could be regarded as representative of bus drivers in general in Buenos Aires. Subjects in the morning shift ($n = 16$) started their shift between 5:30 and 8:30 and finished it between 15:00 and 17:30. Subjects in the afternoon shift ($n = 31$) started their shift between 10:30 and 14:00 and finished it between 20:00 and 22:30.

The large range of the starting and ending times of the shifts obeys to the fact that workers start their shift at different times according to the service demands. Geographic distribution of bus lines was similar for both groups. Before recruitment, the subjects were given detailed information about the procedures, and all subjects gave written informed consent to participate. There was no monetary incentive involved. The study was approved by the ethics committee of Universidad Austral.

Design

This study looked at physiological variables, sleep habits and working conditions in a sample of short distance drivers during seven days.

Day 1: Actigraphs, sleep logs and questionnaires were provided to evaluate sleep habits and working conditions. Actigraphs were placed in the non-dominant wrist and set to start recording in accordance with the worker's shift. Actigraphy and sleep logs were recorded during seven days. Two plastic tubes to collect saliva samples were also provided.

Day 2: Drivers collected salivary samples of cortisol.

Day 3: Heart rate was recorded during working hours. ECG surface electrodes were placed 30 min before the shift started. Resting heart rate variability and alertness were assessed three times at 0, 4 and 8 hours after the beginning of the shift.

Day 8: The subjects returned the questionnaires, sleep logs and actigraphs for data analysis.

Measurements

Demographic and health data

Subjects were asked about demographic and health data including age, height, body weight, smoking habit, regular physical activity habit, medical history of hypertension, dyslipemia, self-perceived psychological stress, diabetes, sleep apnea and heart disease. Body Mass Index (BMI) was calculated (kg/m^2), defining overweight as $\text{BMI} \geq 25 \text{ kg/m}^2$ and obesity as $\text{BMI} \geq 30 \text{ kg/m}^2$.

Working conditions information

Data regarding working conditions included years of driving experience, working hours per day, working days per week and working shift. Also, subjects were asked to indicate to what extent different factors could affect performance (not at all, to a very little extent, to a little extent, to some extent, to a great extent, to a very great extent, completely). Factors included duration of the assignment, route, sleep time, boredom, weather, technical issues, traffic, street conditions and time of the day. Answers were reported as the number and percentage of drivers that identified each factor as affecting performance to a great extent, to a very great extent or completely.

Sleep

Quality of sleep was measured using a Spanish version of the Pittsburgh Sleep Quality Index (PSQI). A score of 6 or more points is associated with bad sleep quality (11, 12). Excessive daytime somnolence was measured using a Spanish version of the Epworth

Sleepiness Scale (ESS). A score of 10 or more points is associated with excessive daytime somnolence (13, 14). Sleep logs were used to determine bedtime and waking up time. Sleep - wake time cycle was determined by wrist accelerometers (MicroMini-Motionloggers® Actigraphs, Ambulatory Monitoring Inc., New York, USA). Wrist actigraphs were used to measure sleep onset (starting time of the first sleep episode after bedtime, as recorded by actigraphy), sleep offset (ending time of the last sleep episode before waking up time, as recorded by actigraphy), time in bed (TIB), time wake after sleep onset (WASO), total sleep time (TST), sleep percentage (%), sleep efficiency (SE%), sleep onset latency (SOL). Drivers were asked to complete the sleep logs and to wear the devices during seven days in the non dominant arm. The actigraphy results were analyzed by the software provided by the manufacturer (Action-W User's Guide, Version 2.4).

Alertness

Reaction time was used for assessing alertness. Reaction time was measured by the Walter Reed palm-held psychomotor vigilance test (palm-PVT) (15). A validated 5-min version of palm-PVT was used (16). This test was run on a Palm-OS-based personal data assistant (Palm Zire 72, Palm Inc, California, USA). Palm-PVT devices use an LCD screen, and visual stimuli are presented on the liquid crystal display. As quickly as possible after the appearance of a visual stimulus the subjects pressed the appropriate response key with the thumb of their dominant hand. Reaction time is the latency between the appearance of the stimulus and the button-push response.

Cortisol

Daily cortisol variations reflect adaptative response to stress; maximum levels are expected in early morning and minimum levels at late afternoon-night. Saliva samples were collected using 10 ml plastic tubes. Participants were instructed not to have breakfast, avoid caffeinated drinks, restrain from smoking, or brush their teeth after waking, as these activities might affect cortisol levels (17). Subjects were originally instructed to collect saliva samples at 7:00 a.m. and 10:00 p.m. (18). However, according to workers reports, the actual saliva collection corresponded to the individual waking time. This was later confirmed by sleep diaries and actigraphy recordings and is reportedly known to reflect more accurately the circadian morning peak of cortisol (19). After the sample was taken, it was stored in the refrigerator until it was processed by ELISA (Elecsys 2010, Roche).

Autonomic activity

Autonomic nervous system activity was assessed by Heart Rate Variability Analysis (HRV). This analysis is based on the fact that the relatively constant heart rate generated by the sinus node, is modulated by several factors that result in a complex heart rate signal. HRV high-frequency (HF) component (0.15 - 0.4.Hz) is related to respiratory sinus arrhythmia and is mediated by the parasympathetic nervous system, whereas the low-frequency (LF) component (0.04 - 0.15 Hz) is related to baroreflex control and depends upon sympathetic and parasympathetic mechanisms. HRV was recorded at 0, 4 and 8 hours at the bus station during a five-min resting period. Quantitative time series analysis was performed on heart rate by evaluating measures of variation of RR interval over time. Among these, RRm (mean duration of RR intervals in ms) quantifies the mean heart rate, SDNN (standard deviation of RR intervals in ms) represents a coarse quantification of overall variability, and RMSSD (square root of the mean squared differences of successive normal RR) measures short-term heart rate variations. Frequency domain (spectral)

measurement of HRV were obtained by Fast Fourier Transform, and included total spectral power (TA, 0-0.4 Hz, ms²), very low frequency power (VLF, < 0.04 Hz, ms²), low frequency power (LF, 0.04-0.15 Hz, ms²), high frequency power (HF, 0.15 - 0.4 Hz, ms²), their percentage values and the LF/HF ratio (20).

Statistical analysis

Normality was assessed by the Kolmogorov-Smirnov test. Numerical variables are expressed as mean \pm standard error and categorical variables are expressed as frequency (%). Differences between shifts for numerical variables were assessed by means of independent-samples T-tests. Categorical variables were compared by a chi-square test. A paired samples T-test was used for comparing cortisol values between day and night. The differences of alertness and HRV along the day were analyzed through a repeated measures analysis of variance (ANOVA). Finally, a general linear model was used for assessing between shifts in alertness, cortisol, and heart rate variability patterns. Significance was assumed when the p value was <0.05. All reported results are 2-tailed.

RESULTS

Demographic data

Table 1 summarizes the demographic data of the sample. Drivers from the morning shift were younger and showed a higher prevalence of overweight. Morning shift started at 7:35 \pm 0:12 (range 5:40-8:39) and finished at 16:18 \pm 0:18 (range 14:54-17:42). Afternoon

shift started at 12:30 \pm 0:12 (range 10:19-13:55) and finished at 21:12 \pm 0:12 (range 19:54-22:42).

Shift characteristics

Table 2 depicts the characteristics of the working shifts. Afternoon shift workers were more experienced than morning shift workers ($p = 0.010$). No differences were found regarding working load per day or per week. Also, the proportion of drivers that identified factors as affecting performance to a great extent or more was similar in both shifts.

Sleep habits

Table 3 shows subjective and objective measurements of sleep habits. Considering the sample as a whole, all the subjects slept less than seven hours according to actigraphy measurements, but only 36% of them were aware of their sleep restriction. Although morning drivers usually wake up three hours earlier than afternoon drivers, they go to sleep only two hours earlier. Subjective sleep time, time in bed, and total sleep time were lower in the morning shift. There were no significant differences between shifts in sleep percentage, sleep efficiency, and sleep latency.

Alertness

Considering the sample as a whole, the mean reaction time on the palm-PVT at time 0 was 0.279 sec \pm 0.005, after 4 hours of work was 0.295 sec \pm 0.007, and after 8 hours of work was 0.301 sec \pm 0.006 ($p = 0.001$). Figure 1 shows the increase in response times according to the working shift. The increase in reaction time was significant in the morning shift ($p = 0.012$), and close to significance in the afternoon shift ($p = 0.066$). No differences were found between shifts in the marginal mean reaction time ($p = 0.753$) nor in the increase in reaction time ($p = 0.481$).

Cortisol response

Figure 2 shows morning and evening cortisol salivary levels. Sleep diaries and actigraphy recordings showed that in the day of cortisol measurement, morning workers reported waking up at 05:37 \pm 00:14 (sleep offset: 05:29 \pm 00:14 hrs) and bedtime at 23:14 \pm 00:16 (sleep onset: 23:35 \pm 00:16), while afternoon workers reported waking up at 08:13 \pm 00:13 (sleep offset: 08:07 \pm 00:13) and bedtime at 00:56 \pm 00:11 (sleep onset: 01:34 \pm 00:15), being the differences between shifts significant ($p < 0.001$). Evening and morning cortisol mean values were similar between shifts. However, morning-evening cortisol difference was higher in drivers who worked in the afternoon shift ($p = 0.023$). Drivers who worked in the morning shift had a 51% \pm 10% non significant reduction of cortisol levels along the day ($p = 0.201$), while in those working in the afternoon shift the reduction was of 74% \pm 4% ($p < 0.001$), with a significant difference between shifts ($p = 0.016$).

Autonomic activity

HRV values are shown in Table 4. In morning drivers, an increase of RMSSD, LF and LF% and a decrease of VLF% along the working day were detected. In afternoon drivers, only mean heart rate showed a significant reduction along the day. RMSSD changes along time were significantly different between shifts (Table 4). HRV marginal means were not different between afternoon and morning drivers (not shown).

DISCUSSION

The main findings of this study are the differences found between morning and afternoon shift in a sample of short-distance bus drivers, regarding the presence of sleep disorders, overweight, decrease in alertness throughout the day and morning/evening salivary cortisol pattern.

Restricted sleep was frequent in our sample; according to actigraphy measurements all the subjects slept less than seven hours. Sleep restriction was more evident in the morning drivers, who slept almost one hour less than afternoon ones. This group showed a significant impairment in reaction time that is known to increase the risk for accidents (21). A recent study in a large sample of commercial drivers found that ESS scores were related to accident risk (22). Driver sleepiness is found typically in healthy people who had had insufficient sleep, and is not necessarily associated with the presence of sleep disorders (23).

Subjective poor quality of sleep, as measured by the PSQI, was present in 66.7% of the sample. Quality of sleep is a subjective measure of sleep disturbance, hard to quantify, multivariable, and with large interindividual variations. Our result could be attributed not only to short sleep at night, but also to sleep fragmentation due sleep-disordered breathing, given the high incidence of overweight and obesity (24). Interestingly, there is a difference between sleep hours referred and sleep hours measured by actigraphy. This condition is characterized by a gross overestimation of sleep, results in a clinically significant excessive daytime sleepiness, which patients were not able to predict (25).

Drivers in the morning shift showed a significant decline in task performance along the working day, while the decline in alertness in afternoon drivers was non-significant. Although there are no studies employing reaction time tests in bus drivers, it was reported that partial sleep restriction and driving time affects alertness and performance of car drivers in simulated driving sessions (26). In real driving environments with relatively short driving duration and time awake, sleep restriction induces important performance decay (27). Thus, the significant increase in reaction time observed in morning workers could be explained by the higher sleep restriction also associated with this shift.

The difference between morning and evening cortisol salivary levels was lower in the morning shift. Cortisol is considered a major indicator of altered physiological states in response to stressful stimulation (28-30). The "flatness" observed in cortisol rhythm may be indicative of a long-term response to chronic stress, since low morning cortisol levels are associated with high levels of psychological stress (31), while high evening levels of cortisol are associated with symptoms of stress and poor self-rated health (32). However, the prevalence of psychological stress, though high, was similar in both shifts. An

alternative explanation could be the shorter sleep found in morning shift workers (33), since the cortisol concentrations upon awakening increase with self-reported sleep duration (34), while the evening cortisol secretion is raised in subjects reporting short sleep (35).

Morning shift drivers had a higher HRV response along the day. Although cardiovascular measures such as heart rate and heart rate variability may serve as early indicators of fatigue (36) our results seems to follow the normal circadian pattern of HRV measures, with a nadir for HF-HRV in the afternoon and an increase of LF relative predominance towards noon (37, 38).

Finally, we found a high prevalence of overweight and obesity, especially in the morning shift. Short sleep duration is associated with a modest increase in future weight gain and incident obesity, as informed in a study that followed 68,000 women during 16 years (39). In addition, a high BMI is a risk factor for snoring and sleep obstructive apnea syndrome (40). Both had been independently related with higher daytime sleepiness and reduced alertness (41, 42). Therefore, the higher BMI observed in the morning shift could also explain the trend towards a higher prevalence of daytime sleepiness and the statistically significant increase of the reaction time along the day observed in morning workers.

Several limitations must be considered for appropriate analysis of the present results. First the sample was unequally distributed in that afternoon workers were older and more experienced than morning workers. It must be noted, however, that age results in a continuously increasing frequency of sleep disturbances (43), decreased reaction time (44), and increased cortisol nocturnal nadir level (45), what would tend to diminish the

differences reported herein. The small sample size prevented us from controlling for these confounders and for performing a multivariate analysis to assess possible associations between variables within each shift, which should be addressed in further studies.

To conclude, this study shows the coexistence of sleep restriction, lower reaction time performance, flattening of cortisol daily variations, and higher overweight prevalence, mainly in the morning working shift of short-distance bus drivers. The differences found between shifts point out to the need of educational strategies to compensate the sleep loss associated with early work schedules. Promoting sleep hygiene and the habit of taking short naps during the day is highly recommended. Organizational policies that encourage the reduction in working hours and the inclusion of rest time schedules may have a favorable impact in drivers' health and a reduction on traffic accidents.

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Table 1. Demographic Data

	Morning shift (n = 16)	Afternoon shift (n = 31)	Total (n = 47)	p
Age (years)	36.19 ± 1.31	41.23 ± 1.65	39.51 ± 1.22	0.021
BMI (kg/m ²)	31.24 ± 1.11	27.62 ± 1.09	28.88 ± 0.84	0.025
Overweight (BMI ≥ 25 kg/m ²)	16 (100%)	23 (76.7%)	39 (84.8%)	0.006
Obesity (BMI ≥ 30 kg/m ²)	9 (56.3%)	9 (30%)	18 (39.1%)	0.086
Hypertension	3 (18.8%)	3 (9.7%)	6 (12.8%)	0.388
Smoking habit	5 (31.3%)	15 (48.4%)	20 (42.6%)	0.263
Dyslipemia	0 (0%)	3 (9.7%)	3 (6.4%)	0.083
Physical activity habit	4 (25%)	12 (38.7%)	16 (34%)	0.344
Diabetes	0 (0%)	0 (0%)	0 (0%)	-
Psychological stress	11 (68.8%)	21 (67.7%)	32 (68.1%)	0.944
Sleep Apnea	2 (12.5%)	0 (0%)	2 (4.3%)	0.111
Heart disease	0 (0%)	0 (0%)	0 (0%)	-

Numerical variables are expressed as media ± standard error. Categorical variables are expressed as frequency (%). Differences between shifts were assessed by means of independent samples T-tests (numerical variables), chi-square tests (categorical variables, except sleep apnea), or Fisher exact test (sleep apnea). BMI: Body Mass Index.

Table 2. Shift characteristics

	Morning shift (n = 16)	Afternoon shift (n = 31)	Total (n = 47)	P
Experience (years)	8.94 ± 1.38	14.26 ± 1.52	12.84 ± 1.22	0.010
Work per Week (days)	5.84 ± 0.11	5.89 ± 0.06	5.87 ± 0.05	0.695
Work per Day (hours)	8.56 ± 0.21	8.67 ± 0.16	8.63 ± 0.13	0.691
Number of drivers that identified factors as affecting performance to a great extent or more				
Time on task	6 (40.0%)	11 (37.9%)	18 (40.0%)	0.897
Route	6 (40.0%)	12 (41.4%)	18 (40.0%)	0.932
Boredom	1 (6.7%)	5 (17.2%)	6 (13.3%)	0.286
Sleepiness	4 (28.6%)	12 (41.4%)	16 (36.4%)	0.419
Weather	3 (20.0%)	8 (27.6%)	11 (24.4%)	0.582
Technical issues	4 (26.7%)	8 (28.6%)	12 (27.3%)	0.898
Traffic	9 (60.0%)	23 (79.3%)	32 (71.1%)	0.181
Time of day	3 (21.4%)	5 (17.2%)	8 (18.2%)	0.748

Numerical variables are expressed as media ± standard error. Categorical variables are expressed as frequency (%). Differences between shifts assessed by means of independent samples T-tests (numerical variables) or chi-square tests (categorical variables).

Table 3. Sleep questionnaires and actigraphy

	Morning Shift (n=16)	Afternoon Shift (n=31)	Total (n = 47)	P
Questionnaires				
Bedtime (hh:mm)	22:42 ± 00:12	00:30 ± 00:12	23:54 ± 00:12	< 0.001
Waking time (hh:mm)	05:30 ± 00:12	08:30 ± 00:12	07:24 ± 00:18	< 0.001
Referred sleep (hrs)	6.8 ± 0.2	8.0 ± 0.3	7.6 ± 0.2	0.002
Referred sleep < 7 hs	9 (56%)	4 (13%)	13 (28%)	0.002
PSQI	6.94 ± 1.03	5.59 ± 0.43	6.07 ± 0.67	0.164
PSQI ≥ 6	10 (55.6%)	15 (53.6%)	25 (54.3%)	0.895
ESS	12.0 ± 0.96	10.42 ± 0.89	10.96 ± 0.67	0.270
ESS ≥10	13 (81.3%)	19 (61.3%)	32 (68.1%)	0.146
Actigraphy				
Bedtime (hh:mm)	23:20 ± 00:17	01:04 ± 00:11	00:29 ± 00:12	< 0.001
Waking time (hh:mm)	05:54 ± 00:16	08:37 ± 00:13	07:43 ± 00:16	< 0.001
Sleep Onset (hh:mm)	23:48 ± 00:17	01:34 ± 00:11	00:58 ± 00:12	< 0.001
Sleep Offset (hh:mm)	05:44 ± 00:17	08:28 ± 00:13	07:33 ± 00:16	< 0.001
TIB (min)	397 ± 20	450 ± 9	432 ± 10	0.010
TST (min)	323 ± 18	381 ± 9	362 ± 10	0.003
TST < 7 hs	15 (100%)	30 (100%)	45 (100%)	-
WASO (min)	38 ± 5	36 ± 3.0	37 ± 3	0.719
SP (%)	82.7 ± 1.5	84.4 ± 1.1	83.8 ± 0.9	0.387
SE (%)	90.6 ± 0.9	91.3 ± 0.7	91.1 ± 0.5	0.556
SE < 90%	6 (40%)	9 (30%)	15 (33.3%)	0.513
SOL (min)	25 ± 4	30 ± 3	28 ± 2	0.390

Numerical variables are expressed as media ± standard error. Categorical variables are expressed as frequency (%). Differences between shifts assessed by means of independent samples T-tests (numerical variables) or chi-square tests (categorical variables). PSQI: Pittsburgh Sleep Quality Index; ESS: Epworth Sleepiness Scale; TIB: time in bed; TST: total sleep time; WASO: wake after sleep onset; SE: sleep efficiency; SP: sleep percentage; SOL: sleep onset latency.

Table 4. Heart rate variability analysis

Time (hours)	Morning Shift (n = 16)				Afternoon Shift (n = 31)				p
	0	4	8	p	0	4	8	p	(time X shift)
RRm (ms)	718 ± 26	762 ± 26	738 ± 25	0.009	721 ± 19	710 ± 21	764 ± 18	0.037	< 0.001
SDNN (ms)	45 ± 5	49 ± 6	47 ± 5	0.630	44 ± 3	50 ± 4	51 ± 2	0.564	0.930
RMSSD (ms)	24 ± 3	32 ± 4	28 ± 4	0.014	27 ± 2	25 ± 2	28 ± 2	0.542	0.006
ln TA (ms ²)	7.4 ± 0.2	7.6 ± 0.3	7.5 ± 0.2	0.760	7.4 ± 0.2	7.6 ± 0.2	7.7 ± 0.1	0.621	0.838
ln VLF (ms ²)	6.8 ± 0.3	6.5 ± 0.3	6.2 ± 0.3	0.378	6.6 ± 0.2	6.8 ± 0.2	6.8 ± 0.1	0.872	0.405
ln LF (ms ²)	6.2 ± 0.2	6.8 ± 0.3	6.8 ± 0.2	0.039	6.4 ± 0.2	6.8 ± 0.2	6.8 ± 0.1	0.383	0.600
ln HF (ms ²)	5.0 ± 0.3	5.6 ± 0.4	5.2 ± 0.3	0.059	5.2 ± 0.2	4.9 ± 0.2	5.2 ± 0.2	0.296	0.016
VLF %	57 ± 4	36 ± 5	33 ± 4	0.010	47 ± 3	45 ± 3	46 ± 4	0.856	0.033
LF %	33 ± 4	48 ± 3	55 ± 4	0.008	41 ± 3	48 ± 3	45 ± 3	0.364	0.082
HF %	10 ± 1	16 ± 2	12 ± 1	0.056	13 ± 1	8 ± 1	9 ± 1	0.132	0.007

Values are expressed as mean ± standard error. Differences assessed by a general lineal model which included time, shifts and time-shift interaction as factors. RRm, mean RR interval; SDNN, standard deviation of all normal RR intervals; RMSSD, square root of the mean squared differences of successive normal RR; TA, total area (total spectral power); VLF, very low frequency power; LF, low frequency power; HF, high frequency power.

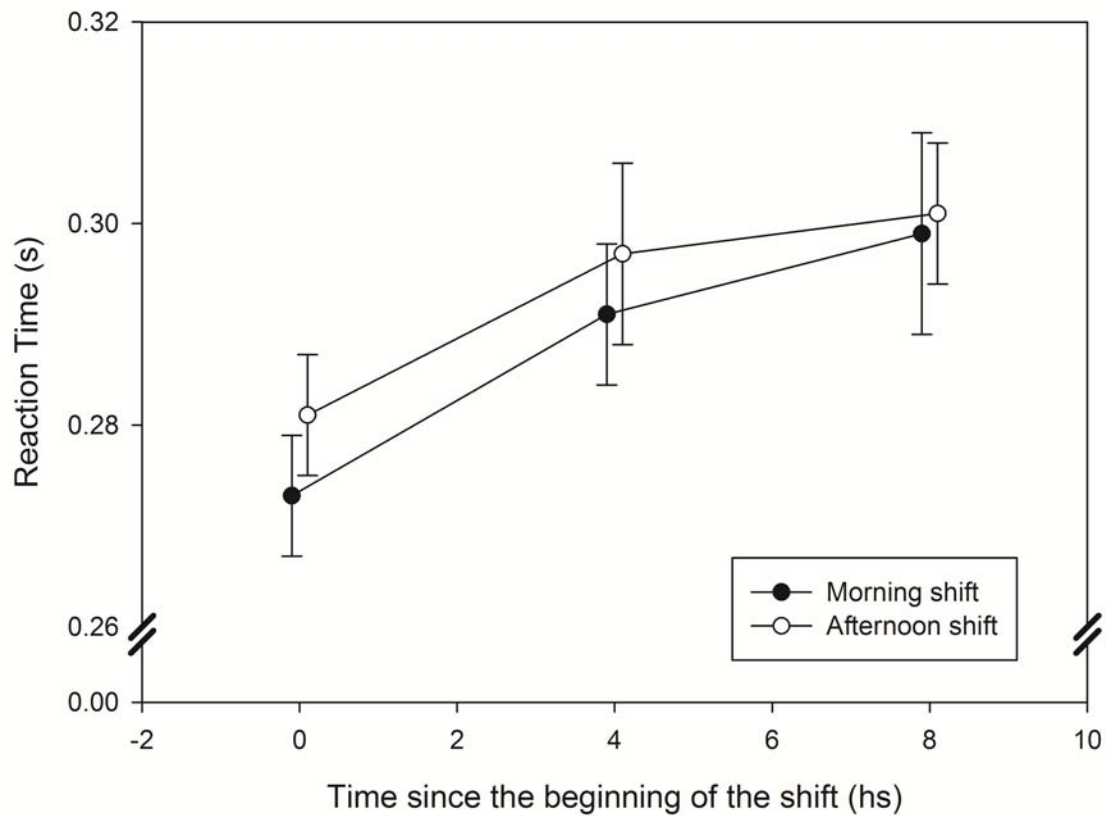


Figure 1. Mean reaction time in the Psychomotor Vigilance Task. The increase in reaction time was significant in the morning shift ($p = 0.012$), and non-significant in the afternoon shift ($p = 0.066$). No differences were found between shifts in the marginal mean reaction time ($p = 0.753$) nor in the decrease in reaction time ($p = 0.481$).

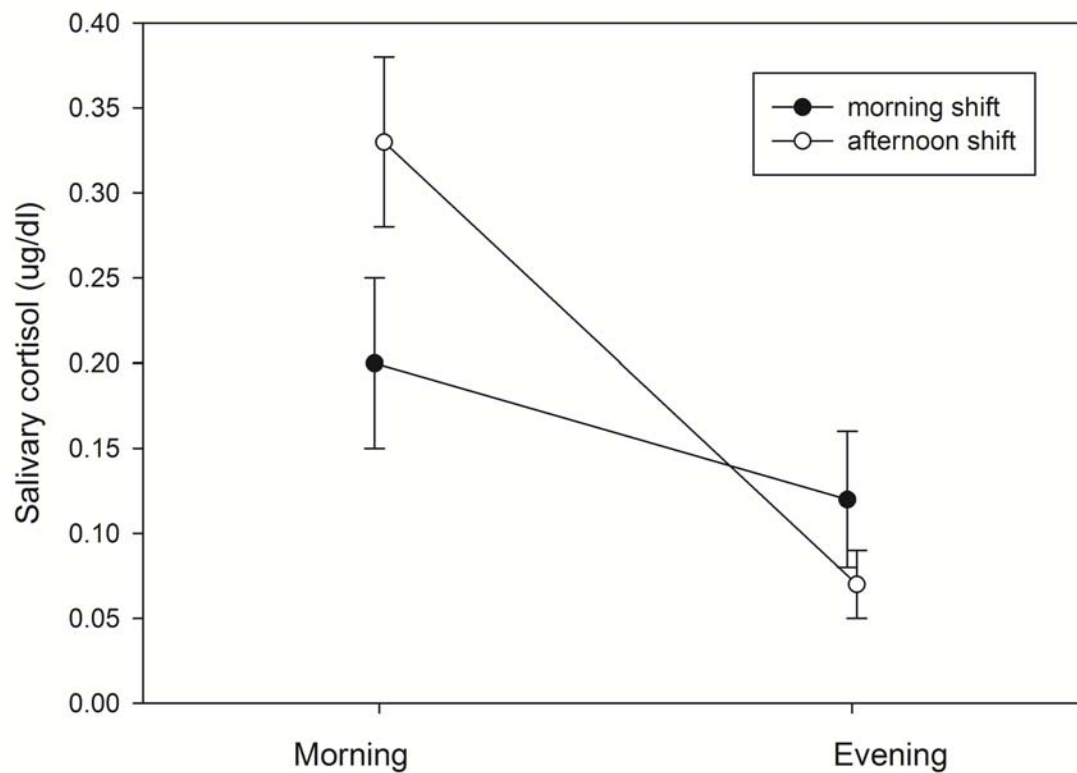


Figure 2. Morning and evening cortisol salivary levels in morning and afternoon shifts. Differences between shifts in morning and evening mean values were non-significant. Morning-night cortisol difference was higher in drivers who worked in the afternoon shift ($p = 0.023$).