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Steroid hormones, vitamin D and melatonin in rapidly rotating shift female hospital workers

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ABSTRACT

Disruption of circadian rhythm caused by night-shift work has been associated with several disorders, including cancer. Health care personnel often works at night to insure the continuity of care. Aim of this study was to evaluate the influence of night-shift work on serum and saliva levels of steroid hormones, vitamin D, and melatonin in hospital female workers. Ninety-seven female hospital workers were recruited: 46 nurses performing clockwise rapid rotating shift schedule on a 5-day cycle, including one night, and 51 day workers. Thirteen steroid hormones and vitamin D were assessed in morning serum samples; cortisol, cortisone and melatonin were assessed in morning and evening saliva samples. We fitted multiple regression models adjusted for age, BMI, sampling month, ovarian cycle phase, and use of oral contraceptives (OC). Rapidly rotating clockwise shift work was associated with increased levels of serum corticosterone, 11-deoxycortisol, dehydroepiandrosterone (DHEA), and androstenedione, and decreased levels of estradiol and vitamin D. OC modulated the association between serum cortisol, corticosterone and 11-deoxycortisol and work shift. The normal circadian phase of salivary melatonin, cortisol and cortisone was not affected by shift work. In female hospital nurses, the clockwise rapid rotating shift schedule increases the level of some hormones, likely associated with stress. No increase of estradiol, nor modification of salivary hormones was observed.

1. Introduction

Night-shift work implies activities that takes place during the normal sleeping hours of the general population. It alters natural light-dark exposure and disrupts normal circadian rhythms. Workers of the health care sector, mostly females, very often perform night-shift work to insure the continuity of care. In Italy, 69 % of tenured health care sector workforce is formed by females, about 450.066, of which 216.206 employed as nurses (Italian Ministry of Health, 2023).

A systematic review on shift work and nurses' health concluded that shift work involves an alteration in psychophysical homeostasis with a decrease in performance; it is an obstacle for social and family relationships, as well as a risk factor for stress sleep disorders, metabolic disorders, diabetes, cardiovascular disorders, and breast cancer (Rosa et al., 2019).

Considering possible health consequences of circadian disruption in shift workers, modification in the homeostasis of endocrine hormones, such as estrogens, testosterone, and other sex hormones, cortisol, prolactin, thyroid hormones, but also modification of melatonin/its metabolite, and vitamin D were investigated (Potter et al., 2016).

The International Agency for the Research on Cancer (IARC) in monograph 124 classified night shift work as "probably carcinogenic to humans" (Group 2 A) (IARC, 2020). In reviewing all studies evaluating the effect of night shift work on blood levels of sex hormones, IARC concluded that there is suggestive evidence of estrogen increase in female night shift workers, with a set of five studies with positive results, including two large studies in nurses (IARC, 2020). This was further supported by a recent Japanese study involving 432 women in which

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more years of night shift work at least once a week during the past 10 years was associated with increased serum free estradiol level (Nagata et al., 2023b).

Cortisol, a hormone associated with stress, has a circadian cycle and reaches the highest levels in the morning, about 30 min after awakening, and gradually decreases during the day (Miller and O'Callaghan, 2002). Cortisol, cortisol rhythm, and cortisol awakening response (CAR) were investigated in relationship to shiftwork. Main findings suggest that shiftwork, especially night shift, significantly disrupts production of cortisol, cortisol rhythm, and CAR; moreover, irregular schedules produce greater disruption than regular shift schedules (reviewed in Grosser et al., 2022).

Melatonin is a hormone with a circadian rhythm regulating the sleep cycle and showing the highest levels around 2:00 a.m.; it was found to be protective against cancer in animal studies (Zawilska et al., 2009). Most human studies support the association between night shift work and decreased level of melatonin (reviewed in IARC, 2020), or other modification of melatonin rhythm such as the deletion of acrophase (Harding et al., 2022).

Vitamin D plays a relevant role for the intestinal absorption of micronutrients that are essential for bone metabolism; it is synthetized in the skin from cholesterol through a chemical reaction that is dependent on sun exposure (specifically UVB radiation). Low levels of vitamin D have been associated with several health disorders. Shift work and depressive symptoms have been investigated in relationship with the mediating effect of vitamin D (Park et al., 2019). A recent review with meta-analysis concluded that shift workers have significant lower levels of serum vitamin D compared to non-shift workers (Martelli et al., 2022); suppling vitamin D to shift working nurses was also suggested (Lehnert et al., 2018).

Aim of the present study was to investigate whether shift work, including night shift, affects the levels of endocrine hormones and vitamin D in female health care workers. A group of female hospital nurses performing a clockwise rapidly rotating shift schedule, including one night, and a matched group of workers performing only day work, were investigated. A panel of thirteen steroid hormones and vitamin D in serum, and salivary cortisol, cortisone and melatonin, were measured applying liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS).

2. Materials and methods

2.1. Study subjects and sample collection

We invited to participate pre-menopausal female hospital workers performing a clockwise rapidly rotating shift schedule on 5-day cycle including one night, or performing only day work. The clockwise rapidly rotating shift schedule includes a morning shift (7:00 – 14:00), an afternoon shift on the second day (14:00 – 21:00), a night shift on the third day (21:00 – 7:00), and, subsequently, two rest days. The two groups were matched for age and length of service.

Inclusion criteria were: age in the range 29–46 years; still in fertile status; Caucasian ethnicity; knowledge of the Italian language good enough to understand the questionnaires and the informed consent form, and, for rapid rotating shift workers, to have worked for at least 1 year on this type of shift schedule. Exclusion criteria were: cancer diagnosis, systemic diseases in acute phase (cardiovascular disorder, brain diseases, or diabetes), use of anti-hypertension and hormone-based medication (excluding oral contraceptives), neurological affection (multiple sclerosis, Alzheimer's disease, Parkinson's disease, mayor depressive disorder, bipolar disorder, schizophrenia, epilepsy), currently pregnant, and obesity (BMI > 30).

Recruitment and sample collection were performed in the hospital Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan. Workers were enrolled during their periodical health surveillance by occupational health physicians. The study was approved by the ethics committee of the hospital; subjects were informed on the aims and methods of the study and signed an informed consent.

Data regarding personal characteristics and habits, including anthropometric characteristics, health status, medication consumption, specifically the use of oral contraceptives (OC), ovarian cycle phase, personal habits, diet, and work characteristics, was collected by a questionnaire administered face-to-face by trained interviewers. Validated tools to evaluate stress at work were self-administered; these included the Standard Shiftwork Index (Barton et al., 1995) and the Effort-reward imbalance questionnaire (Siegrist et al., 2004); the results of this evaluation are briefly summarized here, but are outside the scope of the present paper.

For the determination of serum hormones and vitamin D, a 5 ml blood sample was collected into VACUETTE® Z Serum Sep Clot Activator tubes (Vacuette, Greiner Bio-One Italia S.r.l, Cassina de Pecchi, Italy), by venipuncture; this was performed in the morning, at the end of a night-shift, for the rapid rotating shift workers (about 7:30 am), or at the beginning of a day shift, for the day shift workers (about the same time). Blood samples were kept at room temperature and then centrifuged (15 min at 3000 rpm) within 2 h from collection; serum was separated and stored in polyethylene tubes at −20 °C in the dark until analysis. Two salivary samples, for the analysis of cortisol, cortisone and melatonin, were autonomously collected by study subjects, the first around 11:00 p.m. (evening sample), the day before the blood sampling, and the second around 7:00 a.m. (morning sample), and prior to the blood sample collection. Collection was performed using Salivette® devices (Sarstedt S.r.l., Trezzano sul Naviglio, Italy), according to the manufacturer's instruction. The sample was stored at 4 °C, in the laboratory the oral fluid was extracted from the swab by centrifugation (5 min at 3000 rpm), and stored at −20 °C in the dark until analysis.

2.2. Serum measurements

2.2.1. Steroids assay

To perform the steroids assay, an *in vitro* diagnostic-mass spectrometry (IDV-MS) kit named MassChrom® Steroids in serum/plasma (Chromsystems, Gräfelfing, Germany) was used. Samples were prepared, according to the manufacturer's instructions. Briefly, after two equilibration passages of the 96 SPE well plate, 500 μL of well homogenized serum sample, calibrator, or quality control solution (QC) was placed into each well, and an internal standard solution (containing deuterated analogs of each steroid) was added. The sample was then washed and eluted. The eluate was evaporated under a gentle flow of nitrogen at 45 °C, until completely dryness. After the reconstitution, an aliquot of 40 μL was injected two times onto a liquid chromatography system (Shimadzu, Milano, Italy) to obtain two different chromatographic separations; the analytical column, provided with the kit, was operating at 32 °C. Two different mobile phases, also provided by the manufacturer, were used for the gradient elution of molecules. Two different chromatographic conditions were applied to separate, in Panel 1: aldosterone, cortisol, cortisone, corticosterone, and 11-deoxycortisol, with a run time of 10.5 min; and in Panel 2: dehydroepiandrosterone sulfate (DHEAS), estradiol, testosterone, dehydroepiandrosterone (DHEA), androstenedione, 17-OH-progesterone, dihydrotestosterone (DHT), and progesterone, with a run time of 12.5 min. The chromatographic system was interfaced with a 4500MD mass spectrometer (Sciex, Milano, Italy) equipped with an electrospray ionization source (ESI), operating in positive mode. For calibration, a blank calibrator matrix and six multilevel serum calibrators, provided with the kit, were used. To assess within- and between-run precision and accuracy, three National Institute of Standards and Technology (NIST) certified QC solutions, also provided with the kit, were used. Within-run and between-run precision were < 10 % for all the analytes. The lower limit of quantifications (LLOQ) were: 0.01 μg/L for aldosterone; 1.5 for cortisol; 0.2 μg/L for cortisone; 0.10 μg/L for corticosterone; 0.02 μg/L for 11-deoxycortisol; 75 μg/L for DHEAS; 0.06 μg/L for estradiol; 0.03 μg/L

Table 1
Summary of selected characteristics of study subjects.

Parameter		Statistics	Day work	Rapid rotating shift work	*p*-value
Study subject		N	50	45	-
Age (y)		Mean (min-max)	36.5 (29−46)	35.1 (29−45)	*0.21*
BMI (kg/m^2)		Mean (min-max)	22.2 (18−30)	23.2 (18−29)	*0.12*
Length of service (y)		Mean (min-max)	11.8 (1−26)	10.0 (2−24)	*0.17*
Working time (h/w)		Mean (min-max)	36.4 (18−56)	37.0 (31−48)	*0.52*
Length of work with rapid rotating shift (y)		Mean (min-max)	-	7.4 (1−24)	na
Night shift/year (n)		Mean (min-max)	-	64.5 (50−100)	na
Tobacco smokers	No	N (%)	37 (75)	29 (67)	*0.39*
	Yes		12 (25)	14 (33)	
Alcohol consumption	No	N (%)	39 (80)	34 (81)	*0.38*
	< 35 g/day		9 (18)	5 (12)	
	≥ 35 g/day		1 (2)	3 (7)	
Familiar status	Single	N (%)	18 (37)	16 (36)	*0.91*
	Cohabiting		30 (60)	28 (62)	
Age at marriage (y)		Mean (min-max)	28.6 (23−36)	28.1 (20−39)	*0.75*
Children (n)	0	%	56	89	*0.001*
	1		20	4	
	2		16	7	
	3		8	0	
Ovarian cycle phase	Follicular phase	N (%)	12 (24)	16 (36)	*0.31*
	Luteal phase		22 (44)	15 (33)	
	Ovulation		2 (4)	2 (4)	
	Amenorrhea		2 (4)	0 (0)	
	missing		12 (24)	12 (27)	
Use of oral contraceptives	No	N (%)	32 (64)	26 (58)	*0.45*
	Yes		16 (32)	18 (40)	
	missing		2 (4)	1 (2)	

na= not applicable

for testosterone; 0.04 μg/L for androstenedione; 0.60 μg/L for DHEA; 0.25 μg/L for DHT; 0.06 μg/L for 17-OH-progesterone; 0.03 μg/L for progesterone.

2.2.2. Vitamin D assay

To perform the vitamin D assay, an IVD-MS kit named MassChrom® 25-OH-Vitamin D_3/D_2 in serum/plasma (Chromsystems, Gräfelfing, Germany) was used. Samples were prepared according to the manufacturer's instructions. Briefly, 100 μL of each sample, calibrator, or QC solution was combined with a standard solution containing the deuterated internal standard and a precipitation reagent. The mixture was vortexed, incubated for 10 min at 4 °C, and centrifuged for 10 min using a micro centrifuge (Abbott, Roma, Italy). The supernatant was transferred to an autosampler glass vial, and a 10 μL were injected onto a liquid chromatography system (Shimadzu, Milano, Italy); the system was equipped with a trap column (operating at room temperature) for sample purification and an analytical column (operating at 25 °C) for the peak separation. Mobile phases, provided with the kit, were used for elution in a total run time of 5 min. The chromatographic system was interfaced with a 4500MD mass spectrometer (Sciex, Milano, Italy) equipped with an atmospheric pressure chemical ionization (APCI), operating in positive mode. For calibration, a blank calibrator matrix and three multilevel serum calibrators, provided with the kit, were used. To assess within- and between-run precision and accuracy, two QC solutions, also provided with the kit, were used. Within-run and between-run precision were < 10 %. LLOQ was 3 μg/L.

2.3. Salivary hormones assay

The salivary hormones (melatonin, cortisol, and cortisone) were analyzed by an on-line turbulent flow liquid chromatography/tandem mass spectrometry assay previously described (Fustinoni et al., 2013). Briefly, 500 μL of saliva sample were placed in an autosampler glass vial and a deuterated internal standard solution were added. An aliquot of 50 μL was injected onto a TurboFlow HPLC system (Thermo Scientific, Rodano, Italy) equipped with a turbulent flow liquid chromatography column (Cyclone, 50 mm length, 0.5 mm i.d., Thermo Scientific, Rodano, Italy) for sample purification, and an analytical column (Hypersil Gold, 50 mm length, 2.1 mm i.d., 3 μm particle size, Thermo Scientific, Rodano, Italy) for peak separation. Detection and quantification of analytes were performed using a triple quadrupole mass spectrometer (TSQ Quantum Access, Thermo Scientific, Rodano, Italy) equipped with a heated electrospray ionization (HESI) source. For calibration, a blank calibrator and five multilevel water calibrators were used. To assess within- and between-run precision and accuracy, two QC solutions were used. Within-run and between-run precision were < 10 % for all the analytes. LLOQ were 0.004, 0.3 and 3.0 nmol/L for melatonin, cortisol, and cortisone, respectively.

2.4. Statistical analysis

A value corresponding to one-half of the LLOQ was assigned to measurements <LLOQ.

Levels of hormones, in serum and saliva, and vitamin D were reported as percentage of quantifiable sample, median, minimum, and maximum.

In crude analyses, we used Wilcoxon rank-sum test for comparison between groups and Wilcoxon signed-rank test for paired samples. For estradiol, where in rapid rotating shift workers only 38 % of the data were above the LLOQ level, the chi-square test was used to compare the percentage of detectable samples in each group. For DHT, which had less than 18 % of the data above the LLOQ level in both groups, no further analyses were performed.

In multivariable analyses, we fitted linear regression models adjusted for age (continuous), BMI (Kg/m^2), month of blood sampling, period/type of menstrual cycle (luteal, follicular, ovulatory, amenorrhea), and use of oral contraceptives (no/yes) to evaluate the effect of work-shift type (independent variable, day/night) on the level of serum hormones and vitamin D (dependent variables, all ln-transformed to improve/achieve normal distributions). Logistic models were used for estradiol. We also evaluated effect modification of work-shift type by use of oral contraceptive by including a product term (shift × OC) in the models. When *p* for interaction was < 0.10 we reported results stratified by OC.

Table 2
Results of serum steroid hormones and vitamin D in study subjects, divided according to work-schedule.

Analyte	Statistics	Day work *N = 50*	Rapid rotating shift work *N = 45*	*p*–value
Aldosterone (μg/L)	N > LOQ (%)	100	100	0.21
	Median	0.13	0.13	
	(Min-Max)	(0.01–0.99)	(0.02–0.41)	
Cortisol (μg/L)	N > LOQ (%)	100	100	0.18
	Median	106.0	133.0	
	(Min-Max)	(58.4–392.0)	(37.3–428.0)	
Cortisone (μg/L)	N > LOQ (%)	100	100	0.10
	Median	25.5	23.4	
	(Min-Max)	(13.1–43.2)	(11.7–46.3)	
Corticosterone (μg/L)	N > LOQ (%)	100	100	< 0.001
	Median	1.77	3.02	
	(Min-Max)	(0.50–10.20)	(0.41–17.60)	
11-Deoxycortisol (μg/L)	N > LOQ (%)	100	100	0.003
	Median	0.11	0.25	
	(Min-Max)	(0.03–5.00)	(0.03–1.21)	
DHEAS (μg/L)	N > LOQ (%)	100	100	0.80
	Median	1205	1260	
	(Min-Max)	(381–36660)	(116–3680)	
Testosterone (μg/L)	N > LOQ (%)	100	100	0.40
	Median	0.24	0.22	
	(Min-Max)	(0.06–4.60)	(0.06–1.20)	
Androstenedione (μg/L)	N > LOQ (%)	100	100	0.13
	Median	0.87	0.96	
	(Min-Max)	(0.33–1.66)	(0.23–3.36)	
DHEA (μg/L)	N > LOQ (%)	100	100	0.02
	Median	3.76	4.82	
	(Min-Max)	(1.05–14.80)	(1.38–18.70)	
DHT (μg/L)	N > LOQ (%)	18	9	0.25
	Median	0.13	0.13	
	(Min-Max)	(0.13–0.59)	(0.13–0.42)	
17-OH-Progesterone (μg/L)	N > LOQ (%)	100	96	0.53
	Median	0.42	0.50	
	(Min-Max)	(0.08–3.02)	(0.03–2.66)	
Progesterone (μg/L)	N > LOQ (%)	96	87	0.08
	Median	0.15	0.14	
	(Min-Max)	(0.02–22.90)	(0.02–20.80)	
Vitamin D (μg/L)	N > LOQ (%)	100	100	0.01
	Median	31.9	23.7	
	(Min-Max)	(9.5–54.4)	(8.4–58.7)	
Estradiol (μg/L)	N > LOQ (%)	74	38	0.001
	Median	0.17	0.03	
	(Min-Max)	(0.03–1.10)	(0.03–0.74)	

We expressed the results as percent change using the formula:

$$\text{Change (\%)} = [\exp(\text{slope}) - 1] \times 100$$

For estradiol > LLOQ we expressed the result as [Odd Ratio (OR) – 1] x 100.

For estradiol, we reported OR and 95 % confidence intervals (CI).

Since most variables were missing in 4 workers, we imputed them with multiple imputations with generation of 50 datasets using the "mi" suite of commands in Stata.

Statistical analyses were performed using the SPSS 24.0 package for Windows (SPSS Statistics, IBM Italia) and Stata 18 (StataCorp, 2023).

3. Results

3.1. Study subjects

A total of 97 subjects were recruited: 46 female nurses performing the clockwise rapid rotating shift schedule, and 51 day workers. Two subjects, one in each group, did not complete the protocol and were excluded. Characteristics of the two groups were similar, but the number of children was higher in those performing day work (Table 1). For rapid rotating shift nurses, the mean length service with this type of shift was 7.4 years, with a mean of 64.5 nights per year.

3.2. Stress at work

The tools applied for the evaluation of stress highlighted no difference between rapid rotating shift workers and day workers for several parameters, such as general health conditions, satisfaction at work, effort-reward imbalance, coping strategies, chronic fatigue and the work ability index; significant differences were however found for sleep disturbance, and management of family, social and working life, with the workers performing the rapid rotating shift work showing the poorer conditions.

3.3. Serum steroids and vitamin D

In Table 2, the levels of serum steroid hormones and vitamin D in subjects divided according to their work schedule are depicted. Hormones were quantified in all samples, with the exception of estradiol and DHT that were measurable in 74 % and 18 % of day workers and 38 % and 9 % of rapidly rotating shift workers, respectively. Differences between the two groups were found for corticosterone, 11-deoxycortisol, DHEA, with higher levels in rapidly rotating shift workers, and for vitamin D and estradiol, with lower levels in rapidly rotating shift workers; moreover, suggestive lower cortisone and progesterone levels were observed in rapidly rotating shift workers. When steroid hormones and vitamin D were included in multiple linear regression models as dependent variables, with the type of work schedule as independent

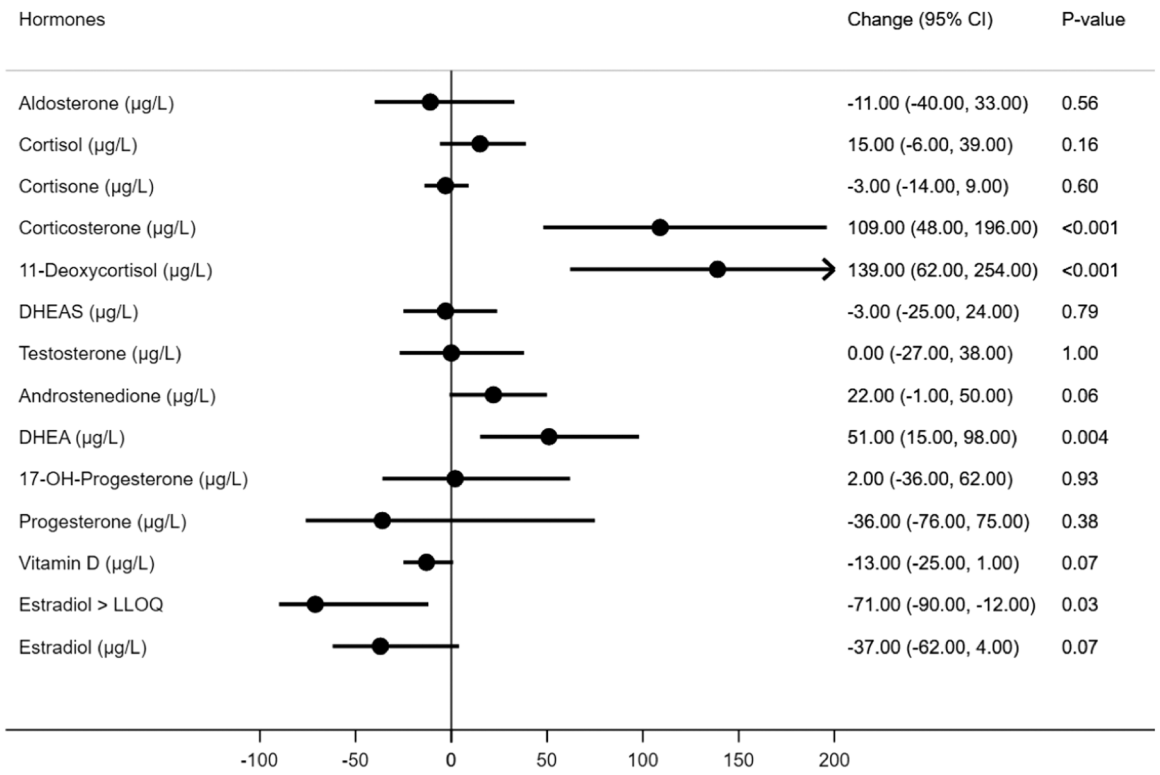

Fig. 1. Forest plot showing the results of the multivariable linear regression models for serum steroid hormones and vitamin D in study subjects: percent change (rapid rotating shift work vs day work) and 95 % confidence interval (CI). For estradiol > LLOQ results refer to (OR – 1) × 100.

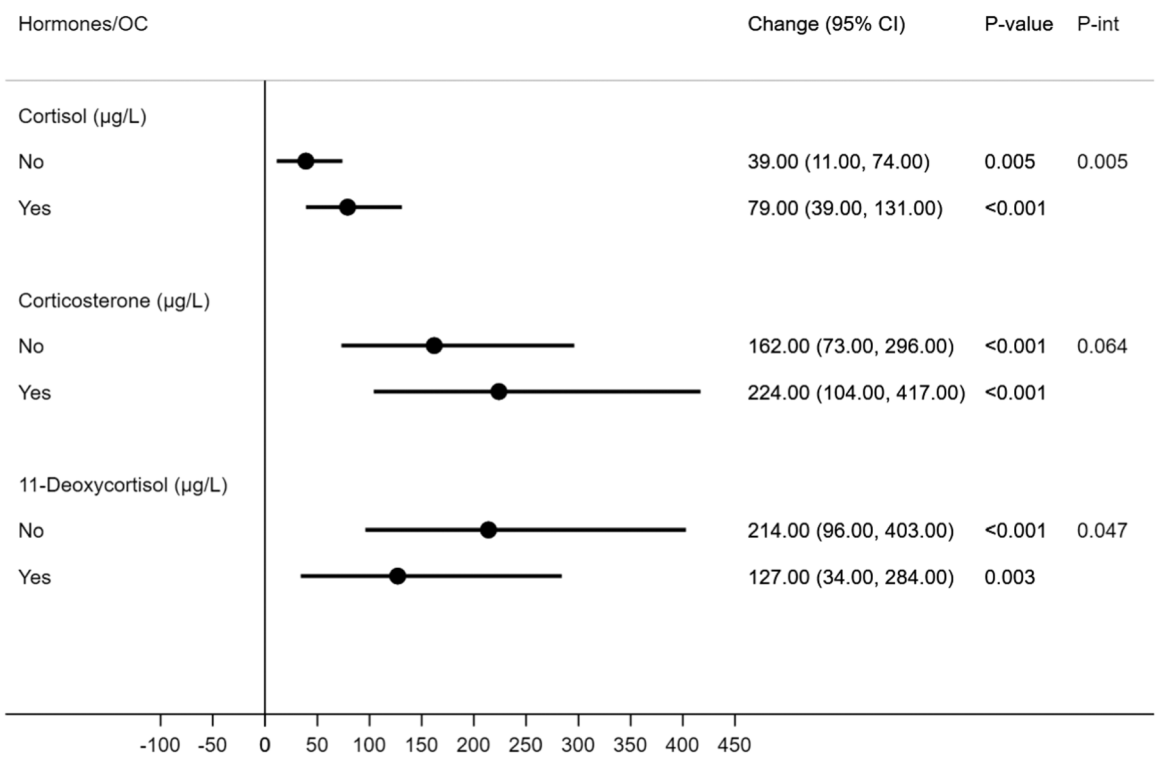

Fig. 2. Forest plot showing the results of the multivariable linear regression models for serum steroid hormones: percent change (rapid rotating shift work vs day work, stratified by use of oral contraceptive, OC) and 95 % confidence interval (CI).

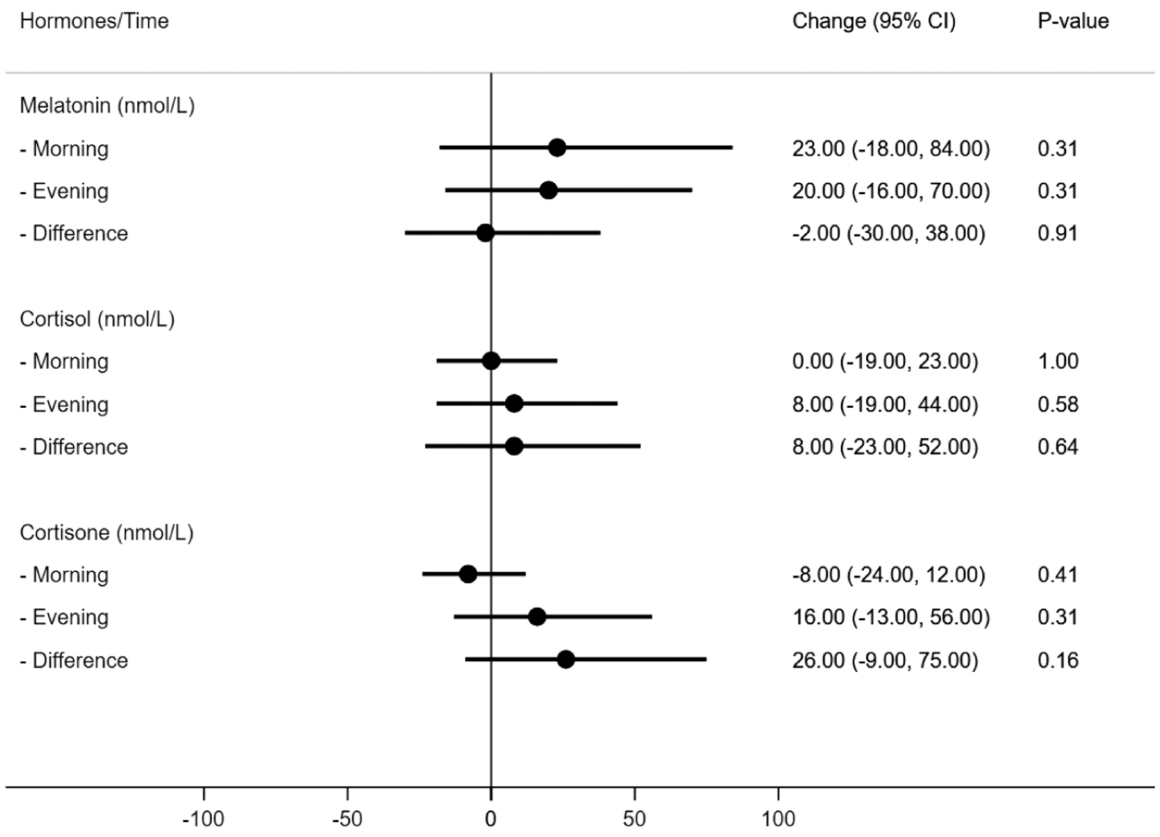

Fig. 3. Forest plot showing the results of the multivariable linear regression models for salivary hormones at different sampling times and their difference in study subjects: percent change (rapid rotating shift work vs day work and 95 % confidence interval, CI).

variable, and the model was adjusted for age, BMI, ovarian cycle phase, use of OC and sampling month, results mostly support those of simple comparisons between groups. In Fig. 1 serum hormones and vitamin D percent change and 95 % confidence interval (CI) in rapidly rotating shift vs. day workers were shown by a Forest plot. In rapidly rotating shift nurses higher levels of corticosterone, with 109 % change (CI 48 – 196 %), 11-deoxycortisol, with 139 % change (CI 62 – 254 %), DHEA with 51 % change (CI 15 – 98 %) and androstenedione with 22 % (CI −1 – 50 %) were found; conversely, lower probability of detectable (> LLOQ) estradiol was observed with −71 % (CI −90 – −12 %) and −37 % (CI −62 – 4 %) percent change when considering the number of quantifiable sample (estradiol > LLOQ) and estradiol concentration (μg/L), respectively. A lower concentration of vitamin D with a −13 % (-25 – 1 %) change in rapid rotating shift nurses was also observed.

In Supplemental Table S1 the concentration of serum steroid hormones and vitamin D are reported according to work schedule and further stratified by oral contraceptives' use. Multiple linear regression models confirmed that OC use enhanced the effect of rapidly rotating work shift in increasing cortisol and corticosterone, while decreased the effect of this shift for 11-deoxycortisol (Fig. 2).

3.4. Salivary hormones

In Supplemental Table S2 the results of salivary hormones melatonin, cortisol and cortisone in samples collected in the morning, in the evening and their differences are reported. No effect of shift work was found for melatonin, while slightly lower levels of morning cortisol and difference between evening and morning levels of cortisol and cortisone were found in rapidly rotating shift workers. Conversely, multiple regression models showed only suggestive higher evening cortisone and increased difference in rapidly rotating shift nurses (Fig. 3).

4. Discussion

In the present study the effect of clockwise rapidly rotating shift schedule was investigated, for the first time, on a panel of biomarkers, including stress and sex hormones, vitamin D and circadian homeostasis. We found some modifications, mostly related to hormones triggered by stress.

After recognition of night shift work as a risk factor for several health disorders (Czeisler et al., 1982), including breast cancer in female workers (IARC, 2020), rapidly rotating shift schedules has been introduced as more protective alternatives to other traditional (e.g. slowly rotating, counterclockwise rotation) shift schemes. When the clockwise rapid rotation shift pattern was compared with the counterclockwise one, it was associated with better characteristics, including: favouring sleep quality and quantity, as well as the alertness and work performance, mitigating circadian interference, and being more compatible with the management of social and family life (Di Muzio et al., 2021; Sallinen and Kecklund, 2010; Shiffer et al., 2018).

The panel of markers analysed in this work encompass 13 steroid hormones in serum in order to include as many classes of steroid hormones as possible: progestogens, cortisol precursors, as progesterone and 17-OH-progesterone; glucocorticoids, as cortisol and its metabolic precursors and/or derivatives, such 11-deoxycortisol and cortisone; mineralocorticoids such as corticosterone and aldosterone; androgens hormones such as DHEA, DHEAS, androstenedione and testosterone; estrogens as estradiol. This has been possible thanks to the availability of a commercial hormones kit to be analysed by liquid chromatography tandem mass spectrometry. This technology, nowadays at its mature state, is allowing specific identification of chemicals through their mass spectrum and sensible detection and accurate quantification, especially in combination with the use of isotopically labelled internal standards. This approach represents the cutting-edge tool for the investigation of

these markers.

The main result of the present study is the higher levels of corticosterone, 11-deoxycortisol, DHEA and androstenedione in serum of nurses performing rapid rotating work shift, including night, in comparison to day workers. This result has never been reported before, possibly because previous studies never investigated these hormones in association with shift work. Considering that cortisol has been associated with stress in several different conditions, and that corticosterone and 11-deoxycortisol share a metabolic proximity with cortisol, being all part of corticosteroid hormones, their increase in nurses working with night shifts seems to be plausible, indicating a higher stress condition than in day shift workers. This is supported by the results of the evaluation of stress performed by the self-administered questionnaire, that showed a higher frequency of sleep disturbance and more difficulties in the management of family, social and working life in nurses performing the rapid rotating shift work. Regarding DHEA, and its metabolic product androstenedione, they are intermediates in the biosynthesis of the androgen and estrogen steroids, having themselves weak androgenic and oestrogenic biological effects. The meaning of their increase in night shift nurses is unclear, also considering that DHEAS, the metabolite of DHEA, is not modified by work shift and that estradiol is lower in night shift nurses.

A second interesting finding of this study is the lower level of estradiol observed in rapidly rotating shift nurses. This does not support results of previous studies showing increased levels of oestrogens in rotating night shift nurses (Bracci et al., 2013; Gómez-Acebo et al., 2015; Nagata et al., 2008a), that were interpreted as possibly related to endocrine disruptions leading to cancer. As a matter of fact, the increased concentration of oestrogens was reported is some studies and not in others (Schernhammer et al., 2006, Langley et al., 2012; Papantoniou et al., 2015), as recently reviewed (IARC, 2020).

The third result of the present study is the (slight) decrease observed in vitamin D; previous studies were suggesting that such effect may be expected in association with night shift work, for the shorter exposure to sunlight in those working at night (Martelli et al., 2022). It should be however noted that our workers, even if involved in night shift, perform such shift for a limited number of nights, on average 64 night per year, and have 4 day shifts (or rest) between night shifts. This suggests that even this type of turnover may be sufficient to cause a slight decrease in vitamin D levels.

A fourth result of the study is the lack of differential changes in salivary hormones associated with wake-sleep cycle. No difference was found in salivary melatonin, cortisol and cortisone and their difference in night vs day workers, recording the maintenance of the physiological differences between morning and evening related to the circadian rhythm of these hormones, and supporting the use of clockwise rapidly rotating work shift. Previous studies showed that this type of rotation is the most effective in preserving sleep quality and quantity; our results support this from the molecular point of view (Shiffer et al., 2018). A recent study investigating salivary cortisol in female hospital workers, found that non-shift and night shift workers has similar diurnal profile during day shift; however, in shift workers on night shift a flattened U-shaped after the post-awakening maximum, and a peak-to-bed slope close to zero was observed (Burek et al., 2024). This points to a modification of cortisol profile during night shift, probably highlighted by the longitudinal design of the study, with the collection of up to eight salivary samples by each participant.

Among strengths of the present study there is the large panel of the investigated hormones and the high quality of analytical data associated with measurements performed by liquid chromatography tandem mass spectrometry. Weaknesses of the study include the limited number of subjects studied and the measurements taken on only one occasion; an enlargement of the study, both in number of subjects and repeated measurements over time, is warranted to further support these findings.

In conclusion, in hospital female nurses, rapidly clockwise rotating shift work, including one night, was associated with increased levels of some steroid hormones (11-deoxycortisol in particular), likely due to stress. Conversely, we did not find increase of estradiol nor disruption of the physiological circadian phase of salivary melatonin, cortisol and cortisone.

Ethics approval

The study was approved by the ethics committee of the hospital Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan. The study was conducted in accordance with all relevant ethical standards. Subjects were informed on the aims and methods of the study and gave their informed consent prior to be included in the study.

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CRediT authorship contribution statement

Frigerio Gianfranco: Formal analysis, Data curation. **Campo Laura:** Writing – review & editing, Methodology, Data curation. **Costa Giovanni:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Crespi Eleonora:** Project administration, Investigation, Conceptualization. **Silvia Fustinoni:** Writing – original draft, Supervision, Resources, Conceptualization. **Mercadante Rosa:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Consonni Dario:** Writing – review & editing, Visualization, Formal analysis, Data curation. **Polledri Elisa:** Writing – original draft, Methodology, Investigation, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxlet.2024.11.013.

Data availability

Data will be made available on request.

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