Salivary cortisol, perceived stress, and metabolic syndrome: a matched case-control study in female shift workers

Short running title: Cortisol, perceived stress and metabolic syndrome

Anderson Garcez, Post-graduate Program in Collective Health, University of Vale do Rio dos Sinos (UNISINOS), Av. Unisinos 920, São Leopoldo, RS, Brazil, 93022-000; Phone: +55-51-35908752; E-mail: adsgarcez@gmail.com

Elisabete Weiderpass, Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; Cancer Registry of Norway, Institute of Population-based Cancer Research, Department of Research, Oslo, Norway; Faculty of Health Sciences, Department of Community Medicine, University of Tromsø, Tromsø, Norway; University of Tromsø, The Arctic University of Norway, Tromsø, Norway; Genetic Epidemiology Group, Folkhälsan Research Center, Helsinki, Finland; Postal address: Box 281 SE-171 77, Stockholm, Sweden; Phone: +47-91-300623; E-mail: elisabete.weiderpass@ki.se

Raquel Canuto, Department of Nutrition, Federal University of Rio Grande do Sul State (UFRGS), St. Ramiro Barcelos, 2400, Porto Alegre, RS, Brazil, 90035-003; Phone: +55-51-33085941; E-mail: raquelcanuto@gmail.com

Sheila Bünecker Lecke, Gynecological Endocrinology Unit, Division of Endocrinology, Hospital de Clínicas de Porto Alegre (HCPA); Department of Diagnostic Methods, Federal University of Health Science of Porto Alegre (UFCSPA), St. Sarmento Leite 245, Porto Alegre, RS, Brazil, 90050-170; Phone: +55-51-33039000; E-mail: s_lecke@hotmail.com

Poli Mara Spritzer, Gynecological Endocrinology Unit, Division of Endocrinology, Hospital de Clínicas de Porto Alegre (HCPA); Laboratory of Molecular Endocrinology, Department of Physiology, Federal University of Rio Grande do Sul State (UFRGS), St. Ramiro Barcelos 2350, Porto Alegre, RS, Brazil, 90035-003; Phone: +55-51-33598027; E-mail: spritzer@ufrgs.br

Marcos Pascoal Pattussi, Post-graduate Program in Collective Health, University of Vale do Rio dos Sinos (UNISINOS), Av. Unisinos 920, São Leopoldo, RS, Brazil, 93022-000; Phone: +55-51-35908752; E-mail: mppattussi@unisinos.br

Maria Teresa Anselmo Olinto, Post-graduate Program in Collective Health, University of Vale do Rio dos Sinos (UNISINOS); Department of Nutrition, Federal University of Health Science of Porto Alegre (UFCSPA); Postal address: Av. Unisinos 920, São Leopoldo, RS, Brazil, 93022-000; Phone: +55-51-35908752; E-mail: mtolinto@gmail.com

Correspondence: Maria Teresa Anselmo Olinto, Post-Graduate Program in Collective Health, University of Vale do Rio dos Sinos, Av. Unisinos 950, C.P. 275, São Leopoldo, RS, Brazil, 93022-000; Phone: +55-51-35901239; Fax: +55-51-35908479; E-mail: mtolinto@gmail.com

Keywords: Cortisol; Perceived stress; Metabolic syndrome; Women; Shift Work.

Summary

Objective Although the pathogenesis of metabolic syndrome (MetS) is complex and multifactorial, there is limited information if psychological factors, such as stress exposure, are involved in the etiology of MetS. Therefore, this study investigated the associations between MetS and cortisol levels and perceived stress levels among women shift workers in Southern Brazil.

Design A matched case-control study was conducted, including 50 cases of MetS and 200 age-matched controls (± 3 yrs, 4 for each case). Salivary cortisol levels were evaluated immediately after waking and one upon returning home from work. Perceived stress levels were measured by the Perceived Stress Scale with 10 items (PSS-10). Multivariate-adjusted associations between MetS and salivary cortisol levels and perceived stress levels were assessed by conditional logistic regression.

Results Means ± standard deviations of salivary cortisol levels were not significantly different between cases and controls either immediately after waking (5.37 ± 4.10 vs. 6.03 ± 5.39 nmol/l; p=0.53) or after work (2.74 ± 2.87 vs. 2.78 ± 2.85 nmol/l; p=0.93). There was no significant difference in perceived stress level between cases and controls (14.2 ± 5.9 vs. 15.5 ± 5.6; p=0.15). No independent association was observed in the multivariate model between MetS and salivary cortisol level or perceived stress level after these exposures were stratified into tertiles.

Conclusions Overall, there was no difference between women with or without MetS in regard to the free salivary cortisol and perceived stress. Our results do not support an association between stress exposure and MetS among women shift workers.

Keywords: Cortisol; Saliva; Perceived stress; Metabolic syndrome; Women; Shift Work.

Introduction

Metabolic syndrome (MetS) is characterized by a combination of different metabolic factors, including hyperglycemia, hypertension, reduced high-density lipoprotein cholesterol (HDL-C), elevated triglycerides, and abdominal obesity [1]. A Joint Interim Statement compiled by different organizations defined MetS as the concomitant presence of three of these five factors [1]. MetS is associated with cardiovascular events and with a higher risk of all-cause mortality [2,3], and these associations are stronger in women than men [4].

The pathogenesis of MetS is complex and multifactorial. However, there is evidence that psychological factors, such as stress exposure, may be involved in the etiology of MetS [5]. Alterations in the activity of the hypothalamic-pituitary-adrenal (HPA) axis lead to increased secretion of stress-related glucocorticoid hormones such as cortisol, which may be involved in the pathogenesis of MetS [6,7]. However, the scientific literature is inconsistent and controversial; some studies have reported positive associations between cortisol and MetS [8-11], whereas others have reported no such association [12-15]. Similar inconsistencies have been observed in relation to perceived stress levels, with some studies reporting an association with MetS [12,16], and others reporting no association [17-19]. Psychological stress can be measured subjectively as perceived stress levels, and objectively as cortisol levels. The perceived stress scale (PSS) is the most widely used tool for measuring the subjective parameter of perceived stress level [20,21], and salivary cortisol level is frequently used as a biological marker of psychological stress [22,23].
Moreover, exposure to certain unfavorable work characteristics may be an important factor in worker's health and health-related outcomes, and shift work, including night shift work, is recognized as a potential risk factor for chronic diseases like MetS [24,25]. Shift work has also been associated with desynchronization of circadian rhythms, sleep problems, and other psychological conditions [26,27]. Based on the plausibility of the evidence regarding these associations for shift work, and given the existing evidence suggesting the association between psychological stress in the development of MetS, the aim of the present study was to investigate the association between MetS and salivary cortisol level and perceived stress level in a sample of female shift workers. So, we hypothesized that levels of salivary cortisol and the susceptibility to perceived stress might account for variation in the occurrence of MetS. We will also examine whether this association is modified by shift work, especially among women who work at night. In doing this, we hope to clarify or

understanding the relationship between MetS and stress exposure in a specific vulnerable population (female shift workers).

Methods

Study population

Female participants from a previous cross-sectional study from a poultry processing plant that operates 24 hours per day in Southern Brazil were used to carry out the present study. The cross-sectional study was carried out in 2010 and aimed to examine the risk factors for metabolic disorders among 902 Brazilian shift workers (65.9% women) [28]. All eligible women were aged ≥18 years and worked 44 hours per week in fixed shifts on the company's assembly line. During the cross-sectional study, data was collected on waist circumference, triglycerides, HDL-C, fasting blood glucose, and blood pressure.

Waist circumference was measured with a non-extendable metric measuring tape (accurate to 1 mm) midway between the lower last rib and the iliac crest, with women standing, feet together, arms slightly apart and along the sides of the body, and abdominal muscles relaxed. Measurements were taken in duplicate for each participant, and the mean value was used for analysis. The cut-off for abdominal obesity was chosen in accordance with the demographics and origins of the study population. Blood samples were collected in the morning from the medial cubital vein after 12 hours of fasting and used to determine triglycerides, HDL-C, and fasting blood glucose. Systolic and diastolic blood pressure was measured on the left upper arm after 5 minutes of rest in the sitting position using an automatic blood pressure monitor (HEM-711INT; OMRON, Tokyo, Japan). Blood pressure measurements were performed in duplicate for each participant, and the mean value was used for analysis.

Study sample

In 2011, female cases of MetS were identified in the cross-sectional study and were included in the present, matched case-control study. Presence of MetS was determined according to the "Harmonizing the Metabolic Syndrome" consensus statement guidelines, which is a Joint Interim Statement of international organizations [1]. A diagnosis of MetS was assigned if three or more of the

following components were present: elevated waist circumference (≥80 cm, cut-off recommended for women of European origin), elevated triglycerides (≥150 mg/dl), reduced HDL-C (<50 mg/dl), elevated fasting blood glucose (≥100 mg/dl or drug treatment of elevated glucose with clinical diagnosis of diabetes mellitus), or elevated blood pressure (systolic ≥130 mm Hg and/or diastolic ≥85 mm Hg, or antihypertensive drug treatment with a clinical diagnosis of hypertension). For each MetS case, four age-matched female controls (± 3 years) were randomly selected from the cross-sectional study. So, the sample size was determined by the total number of cases of MetS (n=68) plus a control group of 272 women without MetS (1 case : 4 matched controls).

Cases and controls were re-interviewed in their homes, using a questionnaire, between January and April 2011. Both women and interviewers were blinded to their case-control status. If a woman in the control group was unable to participate, a new control was selected to replace her. The Ethical Committee of the University of Vale do Rio dos Sinos approved the present study (Project number 3153/2009), and all participants provided signed informed consent forms.

Collection and analysis of salivary cortisol samples

All women were asked to collect two saliva samples during a typical work day: one immediately after waking and one upon returning home from work. The average time between the salivary collections was 12.7 ± 3.1 hrs (12.4 ± 3.2 hrs for cases vs. 12.8 ± 3.1 for controls; p=0.384). Saliva samples were collected with Salivette® (Sarstedt, Rommelsdorf, Germany), a commercially available collection device consisting of a small cotton swab and a standard centrifuge tube. All women were instructed on how to use the kit and the procedure for saliva collection at home during the interview. The swab was rotated in the mouth for at least 2 min and then inserted back into the tube. Women were instructed not to brush their teeth or eat or drink anything for 30 min before sample collection, to record the exact sampling time, and to keep the samples refrigerated (2 to 8°C) until the research group returned to collect them. Once in the laboratory, the samples were centrifuged at 1000 x g for 2 min to provide a clear and non-viscous saliva sample. They were then aliquoted and stored at -40ºC until assayed. Salivary cortisol levels were measured in ng/ml using a commercial immunoassay with chemiluminescence detection (DiaMetra, SRL, Milano, Italy). The higher sensitivity of this assay is 0.33 nmol/l and extreme values were repeated for confirmation. The estimated intra- and interassay

coefficients of variance were below 14%. Before analyses, the continuous data of salivary cortisol levels were transformed via natural logarithms to eliminate skewedness of the distributions.

Measurement of perceived stress level

Psychosocial stress was assessed using the PSS with 10 items (PSS-10) [29,30], which was completed during the interview. The PSS-10 is a global measure of current perceived stress and consists of six negative and four positive items about feelings and thoughts related to events and situations that occurred in the last month. Responses are recorded using a five-point Likert scale (never to very often). After a reverse-score of the positive items, a continuum score from 0 to 40 points is generated. A higher score indicates increased perceived stress level. The PSS-10 showed good internal consistency (reliability). The value of Cronbach's α coefficient was 0.76 with an average inter-item correlation of 0.24.

Assessment of covariates

The questionnaire for the case-control study collected information on demographic, socioeconomic, lifestyle, and occupational characteristics. Age was categorized into three groups (18-29, 30-39, ≥40 years), marital status into two groups (with or without partner), and race/skin color into white or other. Education, measured as years of study completed (<8 years - primary education incomplete, 8-10 years - primary education complete and secondary education incomplete, and >10 years - secondary education complete and higher education incomplete or complete). Economic class was categorized according to the Economic Classification Criterion of the Brazilian Association of Research Companies - ABEP, which estimates the buying power of individuals and families, including the possession of household items and the education level of the head of household. This classification has eight categories in all (A1, A2, B1, B2, C1, C2, D, and E). However, as we did not have any women in the two highest levels (A1 and A2) or in the lowest level (E), we collapsed the categories B1, B2, C1, C2, and D into B (high), C (middle), and D (low). Sleep duration was recorded as hours of sleep per day and dichotomized into ≤5 or >5 hours. Wake time was based on the times provided on the day of sample collection and classified as morning (4:00AM≤hh:hh<12:00AM), afternoon (12:00AM≤hh:hh<8:00PM), or evening (8:00PM≤hh:hh<4:00AM). We also recorded information on

smoking status, alcohol consumption, physical activity and the body mass index (BMI). The BMI was calculated based on self-reported weight and height and categorized as normal, overweight, or obese. Work shift was categorized as day (who started working between 6:00AM and 2:00PM) or night shift (who started working between 6:00PM and 5:00AM, i.e., those who had 90% of their work schedule during hours of darkness). Work sector was classified as more or less fatiguing according to the job characteristics. Information on work shifts and work sectors was collected at the company and confirmed by the women during the interview.

Statistical analyses

Data analysis was performed using Stata version 12.0 (StataCorp LP, College Station, Texas, USA) and SPSS version 22.0 (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics were used to describe the characteristics of the cases and controls and the association between the outcome, exposures, and covariates were examined using the Fisher's exact test for heterogeneity of proportions (categorical data) and the Student's *t*-test for mean comparison (continuous data). Four parameters of salivary cortisol level were analyzed: cortisol level immediately after waking, cortisol level after work, change in cortisol level between waking and after work, and the Area Under the Curve - AUC, calculated from the difference between the two measures of salivary cortisol by the time (in hours) between the two salivary collections [31]. Salivary cortisol levels and the PSS-10 score were examined as continuous variables by the natural logarithms of means and standard deviations (SD) using the Student's *t*-test, the one-way ANOVA test or two-way ANOVA with repeated measures followed by Bonferroni post hoc multiple comparison tests, as appropriate. Due to non-normal distribution, summary measures of the four cortisol parameters and the PSS-10 score were categorized into tertiles to estimate possible linear associations with MetS. Conditional logistic regression for age-matched case-control groups was carried out to test the associations using an unadjusted and a multivariable-adjusted model. Covariates linked to the exposures or the outcome with $p<0.2$ were considered confounding factors and were included in the multivariate model. A two-tailed statistical significance difference was defined at $p<0.05$.

Results

Sixty-eight cases of MetS were considered eligible to participate in this study. Six cases without saliva samples were excluded, and an additional 12 cases were excluded due to loss to follow-up. Thus, the final analytical sample included 50 female MetS cases and 200 female age-matched controls. Using a control-to-case ratio of 4 and a 95% confidence level, the final sample had 80 percent statistical power to detect an odds ratio of 2.50 for the associations between MetS and stress exposure. The average values of the constituent components of MetS were statistically different between cases and controls, which was expected due to the study design. Mean age in the study sample was 36.8 years (SD ± 8.2) and was similar between cases and controls due to matching, and the majority of the women were white (90.8%). No statistically significant differences were observed between cases and controls regarding demographic, socioeconomic, lifestyle, or occupational factors, except for economic class and BMI, where there was an over-representation of cases in the high economic class and with the presence of overweight or obese (Table 1).

Mean (± SD) salivary cortisol levels were not statistically significantly different between cases and controls immediately after waking (mean 5.37 nmol/l; SD ± 4.10 nmol/l vs. mean 6.03 nmol/l ± SD 5.39 nmol/l; p=0.53) or after work (mean 2.74 ± SD 2.87 vs. mean 2.78 ± SD 2.85; p=0.93) nmol/l. There were no differences in changes in cortisol levels, nor in the AUC parameter. PSS-10 scores ranged from 0 to 34 points and showed no significant difference between cases and controls (14.2 ± 5.9 vs. 15.5 ± 5.6; p=0.15) (Table 1).

In the total sample, we found a statistically significant difference across investigated cortisol parameters and PSS-10 score for physical activity, wake time, and shift work. Mean salivary cortisol levels immediately after waking were lower in physically inactive than physically active women; and a borderline significant difference was observed for a lower cortisol levels immediately after waking in night shift workers. Sleep duration <5 hours, wake time in the evening to go to work, and working at night demonstrated an overall significant association with a lower AUC parameter (Table 2). When comparing the cortisol levels in saliva immediately after waking and one upon returning home from work by cases and controls and stratified by their wake time (Figure 1), it was observed a significant difference only in salivary cortisol level immediately after waking among the female shift workers that waking in the evening. In addition, among the controls a significantly lower level of cortisol after work compared to the cortisol levels in saliva immediately after waking was observed, independently

of the wake time, whereas among the cases this difference was observed only among the female shift workers that waking in the morning (Figure 1).

In age-adjusted analyses, none of the investigated cortisol parameters nor the PSS-10 scores demonstrated a statistically significant association with MetS when conditional logistic regression was carried out. MetS cases did not differ from controls in relation to linear trend analysis of these exposures. This lack of association persisted after adjustment for possible confounding factors, including race, economic class, smoking status, physical activity, sleep duration, wake time, and shift work (Table 3).

Discussion

In this case-control study, we found no association between MetS and salivary cortisol levels or perceived stress levels among female shift workers in Southern Brazil. Nonetheless, alterations in AUC parameter seem to be related to shift work and sleep characteristics. In addition, a possible association between MetS and waking salivary cortisol seem to be related to the wake time.

Many previous cross-sectional studies have also suggested no relationship between cortisol levels and MetS, considering MetS a dichotomous rather than a continuous outcome. In an American study among overweight and obese adults in weight loss treatment and healthy volunteers, individuals with MetS were not more likely to have abnormal cortisol results than those without MetS and healthy volunteers [12]. In the Multi-Ethnic Study of Atherosclerosis (MESA study), MetS was not associated with 18 measures of salivary cortisol taken at different points in time among healthy adults in the United States [13]. In a Korean study, cortisol levels were not associated with MetS in women after adjustment for socioeconomic status, smoking, alcohol drinking, and daily physical activity [15]. Similarly, in an Iranian case-control study of adult patients with MetS from a diabetes clinic and healthy controls, the cortisol levels of controls did not differ significantly from those of MetS cases after multiple adjustments [14].

Moreover, the results of the above-mentioned studies were similar to ours even though they used different definitions of MetS and considered different cortisol parameters than we did. Some studies used the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria for MetS [12-14] and/or having received medical treatment for diabetes, hypertension, or elevated triglycerides [12], whereas others used NCEP-ATP III Asian guidelines [15]. These studies

considered various salivary cortisol measures: including measures over 3 days [13], 24 h urine cortisol excretion, bedtime salivary cortisol and the 1 mg dexamethasone suppression test [12], and fasting plasma cortisol [14,15].

Salivary cortisol would be dysregulated in individuals with MetS. A clinical review showed that alterations in the HPA axis, and consequent changes in glucocorticoids, may contribute to the pathogenesis of MetS [6]. As previous findings have been inconsistent, it is unclear whether changes in cortisol level are a cause or a result of MetS; these conflicting results about HPA axis function and MetS may be due to the use of different study designs, differences in age and sex across study samples, or the lack of adjustment for important confounding factors, such as sleep behaviors, in statistical analyses. Moreover, many studies have assessed cortisol levels at different times of day in plasma, hair, or urine, rather than in saliva. Finally, some studies analyzed these associations in specific populations, such as individuals with diabetes, and/or overweight or obese individuals.

The present study explored the association between perceived stress and MetS using a validated version of the PSS-10. In agreement with or results, previous studies showed no statistically significant difference in perceived stress between individuals with and without MetS, regardless of the definition of MetS or the instruments used to measure perceived stress. For example, a cross-sectional analysis reported no association between the PSS with 14 items (PSS-14) and the prevalence of MetS at baseline in a population-based prospective cohort study of middle-aged healthy women (Healthy Women Study – HWS), or between the PSS-14 and the risk of developing MetS [18]. In another study, the PSS-14 score was not associated with MetS among a sample of male law enforcement officers from the Iowa Department of Public Safety [19]. Similar results were observed in other studies using a specific self-perceived psychological stress questionnaire and after adjustment for lifestyle factors and other mediating biological risk factors [17,32].

Abnormal work schedules, especially night work, may influence health-related outcomes via a circadian stress pathway [27]. We hypothesized that shift work could be an important factor involved in the relationship between psychological stress and MetS. Working atypical shifts may have important impacts on sleep and circadian rhythms [26], as well as on the risk of MetS [24]. In this direction, our results appointed a possible association between MetS and the cortisol level in saliva immediately after waking according to the wake time. On the other hand, a review study indicated no association between shift work and MetS when confounders such as sleep duration were taken into

account [33]. In addition, the diurnal rhythm of cortisol (i.e., high cortisol levels in the morning and low levels in the evening), would be altered by shift work [27]. In our study, women who worked the night shift showed differences in some of the investigated cortisol parameters. The AUC parameter was generally lower in night shift workers than day shift workers, which is consistent with previous findings [34]. Similar results were observed among women who slept <5 hours per day and those who woke in the evening to go to work, two important characteristics associated with shift work that affect HPA axis activity.

To our knowledge, this is the first study to evaluate the relationship between psychological stress exposure and MetS among female shift workers using a robust case-control study design with sex restriction, matched by age, and controlling for potential confounding factors. This design is appropriate for investigating health-related events with a long exposure time and is also advantageous because of its low cost and its utility in studying the association of particular exposures on a predetermined outcome. The main strength of this study included the use of appropriate criteria for defining MetS cases[1], and the use of free salivary cortisol as a biomarker of psychological stress which represents the biologically active hormone fraction [22]; taking into account an appropriate and standardized control of sampling and laboratory analysis of saliva. In comparison with serum, urine, or hair tests, cortisol measurement in saliva has been increasingly incorporated into epidemiological research for psychological stress exposure diagnosis [35]. Additionally, we used the PSS-10, a popular and validated questionnaire, to measure the self-reported perceived stress level [20,21]. This instrument demonstrated adequate psychometric properties, and the reliability analysis showed alpha coefficients similar to those reported in the original study [30], and those observed in Brazilian adults [36]. Finally, considering that psychological stress measurements may differ between the sexes [22], the restriction of our study sample to women can also be considered an important strength of this study.

Our study has some major limitations. First, the determination of causality could be limited because we used prevalent cases of MetS rather than incident cases. Second, an over-matching/over-controlling bias cannot be ruled out, and if present it could make the detection of associations between psychological stress markers and MetS more difficult. Third, the cases and controls were not matched by work shift, limiting our matching procedure in this study. A fourth limitation is that the cortisol sampling protocol was sparse, with only two samples per day on a single workday; in addition, the

lack of assessment of salivary cortisol at bedtime could have limited our findings, taking in to account the importance of this measure to assess the circadian rhythm of cortisol and response to stress exposure [37,38]. A fifth limitation refers to the use of basal cortisol as a general measure of psychological stress level. The final limitation derives from the relatively small sample size, particularly the small number of MetS cases, which may have limited the statistical power of this study; however, the selection and inclusion of four age-matched controls for each MetS case may have attenuated this limitation.

Conclusions

In conclusion, the present study has shown that among female shift workers in Southern Brazil, stress exposure, estimated objectively by cortisol level and subjectively by self-reported perceived stress level, was not statistically significantly associated with MetS. We found no evidence of differences in salivary cortisol levels and perceived stress levels between women with and without MetS. Nonetheless, alterations in the AUC parameter seem to be related to shift work and sleep characteristics and a possible association between MetS and waking salivary cortisol seem to be related to the wake time. Our findings should be cautiously extrapolated to rotating-shift workers or other populations. Future epidemiological studies of shift workers will be needed to confirm our findings or to assess whether salivary cortisol or perceived stress are associated with the development of MetS, taking into account lifestyle factors and job characteristics in order to improve our understanding of the complexities of this relationship.

References

1. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC, Jr., International Diabetes Federation Task Force on E, Prevention, Hational Heart L, Blood I, American Heart A, World Heart F, International Atherosclerosis S, International Association for the Study of O. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009; 120: 1640-1645

2. Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, Rinfret S, Schiffrin EL, Eisenberg MJ. The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. J Am Coll Cardiol 2010; 56: 1113-1132

3. Wu SH, Liu Z, Ho SC. Metabolic syndrome and all-cause mortality: a meta-analysis of prospective cohort studies. Eur J Epidemiol 2010; 25: 375-384

4. Kragelund C, Kober L, Faber J, Steffensen R, Hildebrandt P. Metabolic syndrome and mortality in stable coronary heart disease: relation to gender. Int J Cardiol 2007; 121: 62-67

5. Rosmond R. Role of stress in the pathogenesis of the metabolic syndrome. Psychoneuroendocrinology 2005; 30: 1-10

6. Anagnostis P, Athyros VG, Tziomalos K, Karagiannis A, Mikhailidis DP. Clinical review: The pathogenetic role of cortisol in the metabolic syndrome: a hypothesis. J Clin Endocrinol Metab 2009; 94: 2692-2701

7. Pasquali R, Vicennati V, Cacciari M, Pagotto U. The hypothalamic-pituitary-adrenal axis activity in obesity and the metabolic syndrome. Ann N Y Acad Sci 2006; 1083: 111-128

8. Almadi T, Cathers I, Chow CM. Associations among work-related stress, cortisol, inflammation, and metabolic syndrome. Psychophysiology 2013; 50: 821-830

9. Jang YM, Lee EJ, Kim DL, Kim SK, Song KH. The Association between Midnight Salivary Cortisol and Metabolic Syndrome in Korean Adults. Diabetes Metab J 2012; 36: 245-250

10. Stalder T, Kirschbaum C, Alexander N, Bornstein SR, Gao W, Miller R, Stark S, Bosch JA, Fischer JE. Cortisol in hair and the metabolic syndrome. J Clin Endocrinol Metab 2013; 98: 2573-2580

11. Ward AM, Fall CH, Stein CE, Kumaran K, Veena SR, Wood PJ, Syddall HE, Phillips DI. Cortisol and the metabolic syndrome in South Asians. Clin Endocrinol (Oxf) 2003; 58: 500-505

12. Abraham SB, Rubino D, Sinaii N, Ramsey S, Nieman LK. Cortisol, obesity, and the metabolic syndrome: a cross-sectional study of obese subjects and review of the literature. Obesity (Silver Spring) 2013; 21: E105-117

13. DeSantis AS, DiezRoux AV, Hajat A, Golden SH, Jenny NS, Sanchez BN, Shea S, Seeman TE. Associations of salivary cortisol levels with metabolic syndrome and its components: the multi-ethnic study of atherosclerosis. J Clin Endocrinol Metab 2011; 96: 3483-3492

14. Esteghamati A, Morteza A, Khalilzadeh O, Noshad S, Novin L, Nakhjavani M. Association of serum cortisol levels with parameters of metabolic syndrome in men and women. Clin Invest Med 2011; 34: E131-137

15. Park SB, Blumenthal JA, Lee SY, Georgiades A. Association of cortisol and the metabolic syndrome in Korean men and women. J Korean Med Sci 2011; 26: 914-918

16. Räikkönen K, Matthews KA, Kuller LH. Depressive symptoms and stressful life events predict metabolic syndrome among middle-aged women: a comparison of World Health Organization, Adult Treatment Panel III, and International Diabetes Foundation definitions. Diabetes Care 2007; 30: 872-877

17. Frisman GH, Kristenson M. Psychosocial status and health related quality of life in relation to the metabolic syndrome in a Swedish middle-aged population. Eur J Cardiovasc Nurs 2009; 8: 207-215

18. Räikkönen K, Matthews KA, Kuller LH. The relationship between psychological risk attributes and the metabolic syndrome in healthy women: antecedent or consequence? Metabolism 2002; 51: 1573-1577

19. Yoo HL, Eisenmann JC, Franke WD. Independent and combined influence of physical activity and perceived stress on the metabolic syndrome in male law enforcement officers. J Occup Environ Med 2009; 51: 46-53

20. Lee EH. Review of the psychometric evidence of the perceived stress scale. Asian Nurs Res (Korean Soc Nurs Sci) 2012; 6: 121-127

21. Taylor JM. Psychometric analysis of the Ten-Item Perceived Stress Scale. Psychol Assess 2015; 27: 90-101

22. Hellhammer DH, Wust S, Kudielka BM. Salivary cortisol as a biomarker in stress research. Psychoneuroendocrinology 2009; 34: 163-171

23. Levine A, Zagoory-Sharon O, Feldman R, Lewis JG, Weller A. Measuring cortisol in human psychobiological studies. Physiol Behav 2007; 90: 43-53

24. Wang F, Zhang L, Zhang Y, Zhang B, He Y, Xie S, Li M, Miao X, Chan EY, Tang JL, Wong MC, Li Z, Yu IT, Tse LA. Meta-analysis on night shift work and risk of metabolic syndrome. Obes Rev 2014; 15: 709-720

25. Wang XS, Armstrong ME, Cairns BJ, Key TJ, Travis RC. Shift work and chronic disease: the epidemiological evidence. Occup Med (Lond) 2011; 61: 78-89

26. Boivin DB, Boudreau P. Impacts of shift work on sleep and circadian rhythms. Pathol Biol (Paris) 2014; 62: 292-301

27. Puttonen S, Harma M, Hublin C. Shift work and cardiovascular disease - pathways from circadian stress to morbidity. Scand J Work Environ Health 2010; 36: 96-108

28. Canuto R, Pattussi MP, Macagnan JB, Henn RL, Olinto MT. Metabolic syndrome in fixed-shift workers. Rev Saúde Pública 2015; 49: 30

29. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. J Health Soc Behav 1983; 24: 385-396

30. Cohen S, Williamsom GM. Perceived Stress in a Probability Sample of United States. In: Spacapan S, Oskamp S eds, The social psychology of health: claremont symposium on applied social psychology. Newbury Park, CA: Sage; 1988: 31-67

31. Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 2003; 28: 916-931

32. Ortega-Montiel J, Posadas-Romero C, Ocampo-Arcos W, Medina-Urrutia A, Cardoso-Saldana G, Jorge-Galarza E, Posadas-Sanchez R. Self-perceived stress is associated with adiposity and atherosclerosis. The GEA Study. BMC Public Health 2015; 15: 780

33. Canuto R, Garcez AS, Olinto MT. Metabolic syndrome and shift work: a systematic review. Sleep Med Rev 2013; 17: 425-431

34. Kudielka BM, Buchtal J, Uhde A, Wust S. Circadian cortisol profiles and psychological self-reports in shift workers with and without recent change in the shift rotation system. Biol Psychol 2007; 74: 92-103

35. Adam EK, Kumari M. Assessing salivary cortisol in large-scale, epidemiological research. Psychoneuroendocrinology 2009; 34: 1423-1436

36. Reis RS, Hino AA, Rodrigues-Añez CR. Perceived stress scale: reliability and validity study in Brazil. J Health Psychol 2010; 15: 107-114

37. Raff H, Raff JL, Duthie EH, Wilson CR, Sasse EA, Rudman I, Mattson D. Elevated salivary cortisol in the evening in healthy elderly men and women: correlation with bone mineral density. J Gerontol A Biol Sci Med Sci 1999; 54: M479-483

38. Dahlgren A, Kecklund G, Theorell T, Akerstedt T. Day-to-day variation in saliva cortisol--relation with sleep, stress and self-rated health. Biol Psychol 2009; 82: 149-155

Table 1. General characteristics of cases of metabolic syndrome (MetS) and matched controls in female shift workers (n=250), Southern Brazil, 2011

Characteristics	Total Sample (n=250)	Cases (n=50)	Controls (n=200)	*p*-value*
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	
Age (years)	36.8 ± 8.2	37.3 ± 8.7	36.6 ± 8.1	Matched
Determinants of MetS				
Waist circumference (cm)	87.6 ± 10.8	94.8 ± 8.8	85.8 ± 10.5	<0.001
Triglycerides (mg/dl)	107.9 ± 35.7	142.4 ± 49.1	99.3 ± 25.0	<0.001
HDL-cholesterol (mg/dl)	53.3 ± 10.4	45.6 ± 7.9	55.2 ± 10.1	<0.001
Glucose level (mg/dl)	80.9 ± 9.7	84.3 ± 15.1	80.2 ± 7.7	0.006
Systolic BP (mm Hg)	120.9 ± 14.7	129.8 ± 15.2	118.7 ± 13.8	<0.001
Diastolic BP (mm Hg)	75.8 ± 11.3	83.2 ± 10.0	73.9 ± 10.9	<0.001
Cortisol parameters				
Cortisol level immediately after waking (nmol/l)	5.90 ± 5.16	5.37 ± 4.10	6.03 ± 5.39	0.57
Cortisol level after work (nmol/l)	2.77 ± 2.85	2.74 ± 2.87	2.78 ± 2.85	0.93
Cortisol change (nmol/l)	-3.13 ± 6.39	-2.63 ± 5.93	-3.25 ± 6.51	0.77
Area Under Curve - AUC (nmol/l/h)	55.0 ± 37.8	51.5 ± 30.4	55.8 ± 39.4	0.58
PSS-10 score (0 - 40 points)	15.2 ± 5.7	14.2 ± 5.9	15.5 ± 5.6	0.15
	n (%)	**n (%)**	**n (%)**	
Race (skin color)				0.42
White	227 (90.8)	44 (88.0)	183 (91.5)	
Other	23 (9.2)	6 (12.0)	17 (8.5)	
Marital status				0.51
Without partner	39 (15.6)	6 (12.0)	33 (16.5)	
With partner	211 (84.4)	44 (88.0)	167 (83.5)	
Education (years)				0.27
<8	105 (42.0)	33 (46.5)	79 (39.5)	
8-10	49 (19.6)	13 (18.3)	40 (20.0)	
>10	96 (38.4)	25 (35.2)	81 (40.5)	
Economic class (ABEP scale)				0.03
B (high)	37 (14.8)	11 (22.0)	26 (13.0)	
C (middle)	204 (81.6)	35 (70.0)	169 (84.5)	
D (low)	9 (3.6)	4 (8.0)	5 (2.5)	
Smoking status				0.24
Nonsmoker	224 (89.6)	42 (84.0)	182 (91.0)	
Former smoker	20 (8.0)	6 (12.0)	14 (7.0)	
Smoker	6 (2.4)	2 (4.0)	4 (2.0)	
Alcohol consumption				0.87
Non-drinkers	116 (46.4)	24 (48.0)	92 (46.0)	
Drinkers	134 (53.6)	26 (52.0)	108 (54.0)	
Physical activity				0.61
Active	80 (32.0)	14 (28.0)	66 (33.0)	
Inactive	170 (68.0)	36 (72.0)	134 (67.0)	
Body mass index (BMI)				0.001
Normal (BMI <25 kg/m^2)	130 (52.0)	14 (28.0)	116 (58.0)	
Overweight (25 kg/m^2≤ BMI <30 kg/m^2)	85 (34.0)	25 (50.0)	60 (30.0)	
Obese (BMI ≥30 kg/m^2)	35 (14.0)	11 (22.0)	24 (12.0)	
Sleep duration (hours/day)				0.56
≤5 h	53 (21.2)	12 (24.0)	41 (20.5)	
>5 h	197 (78.8)	38 (76.0)	159 (79.5)	
Wake time				0.97
Morning (4:00AM≤hh:hh<12:00AM)	134 (53.6)	26 (52.0)	108 (54.0)	
Afternoon (12:00AM≤hh:hh<8:00PM)	35 (14.0)	7 (14.0)	28 (14.0)	
Evening (8:00PM≤hh:hh<4:00AM)	81 (32.4)	17 (34.0)	64 (32.0)	
Work shift				0.41
Day	89 (35.6)	15 (30.0)	74 (37.0)	
Night	161 (64.4)	35 (70.0)	126 (63.0)	
Work sector (fatigue)				0.60
Less fatiguing	75 (30.0)	13 (26.0)	62 (31.0)	
More fatiguing	175 (70.0)	37 (74.0)	138 (69.0)	

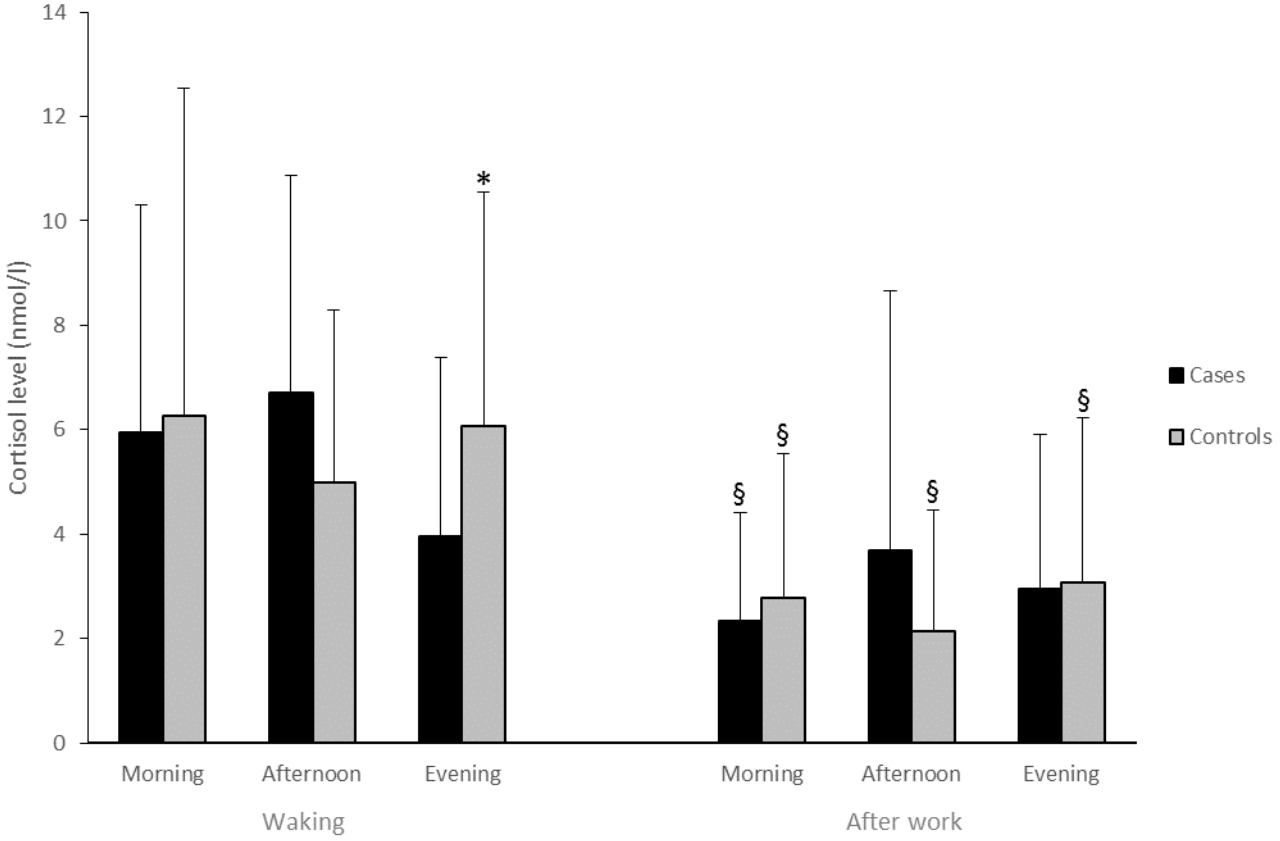
Table 2. Summary of salivary cortisol parameters and perceived stress values according to demographic, socioeconomic, lifestyle and occupational factors in female shift workers (n=250), Southern Brazil, 2011

Characteristics	Cortisol level immediately after waking (nmol/l)	*p*-value	Cortisol level after work (nmol/l)	*p*-value	Change in cortisol level (nmol/l)	*p*-value	Area Under Curve AUC (nmol/l/h)	*p*-value	Perceived stress PSS-10 score	*p*-value
Age (years)		0.61		0.96		0.21		0.80		0.16
18 – 30	5.44 ± 4.51		2.97 ± 3.20		-2.48 ± 6.23		51.9 ± 34.7		16.2 ± 6.3	
31 – 40	5.73 ± 4.44		2.65 ± 2.75		-3.08 ± 5.78		52.6 ± 30.9		15.0 ± 5.3	
>40	6.42 ± 6.19		2.72 ± 2.64		-3.70 ± 7.05		59.7 ± 45.2		14.6 ± 5.5	
Race (skin color)		0.26		0.72		0.89		0.56		0.16
White	5.82 ± 5.16		2.77 ± 2.89		-3.05 ± 6.41		54.6 ± 37.2		15.1 ± 5.8	
Other	6.67 ± 5.25		2.79 ± 2.50		-3.88 ± 6.27		58.9 ± 43.6		16.8 ± 5.1	
Marital status		0.54		0.22		0.38		0.46		0.59
Without partner	4.93 ± 3.63		2.27 ± 1.94		-2.66 ± 4.75		47.4 ± 24.2		14.8 ± 6.3	
With partner	6.08 ± 5.39		2.86 ± 2.98		-3.21 ± 6.66		56.4 ± 39.7		15.3 ± 5.6	
Education (years)		0.60		0.77		0.97		0.23		0.65
<8	6.04 ± 4.64		2.58 ± 2.53		-3.46 ± 5.74		57.4 ± 34.2		14.8 ± 5.3	
8-10	5.62 ± 6.63		2.89 ± 2.82		-2.73 ± 7.49		51.3 ± 47.1		15.3 ± 6.0	
>10	5.88 ± 4.90		2.92 ± 3.18		-2.96 ± 6.51		54.2 ± 36.3		15.6 ± 6.0	
Economic class		0.19		0.59		0.69		0.27		0.42
B (high)	5.99 ± 5.21		2.99 ± 2.71		-3.00 ± 6.20		55.2 ± 32.7		14.4 ± 5.5	
C (middle)	5.97 ± 5.20		2.75 ± 2.89		-3.23 ± 6.47		55.6 ± 38.9		15.3 ± 5.8	
D (low)	3.80 ± 4.04		2.44 ± 2.61		-1.36 ± 6.66		39.5 ± 28.1		17.1 ± 4.6	
Smoking status		0.42		0.15		0.29		0.13		0.10
Nonsmoker	5.94 ± 5.18		2.82 ± 2.90		-3.12 ± 6.41		55.2 ± 37.2		15.0 ± 5.6	
Former smoker	5.52 ± 5.09		2.68 ± 2.63		-2.84 ± 6.60		54.6 ± 41.4		16.0 ± 6.8	
Smoker	5.50 ± 5.59		1.27 ± 0.81		-4.23 ± 5.59		45.9 ± 52.1		19.8 ± 6.3	
Alcohol consumption		0.94		0.23		0.48		0.54		0.85
Non-drinkers	5.87 ± 5.27		2.49 ± 2.16		-3.36 ± 5.98		53.3 ± 36.7		15.1 ± 6.3	
Drinkers	5.92 ± 5.08		3.01 ± 3.32		-2.91 ± 6.74		56.4 ± 38.8		15.3 ± 5.2	
Physical activity		0.04		0.75		0.90		0.18		0.84
Active	6.72 ± 4.78		2.79 ± 2.64		-3.93 ± 5.97		58.3 ± 32.9		15.1 ± 5.9	
Inactive	5.51 ± 5.30		2.76 ± 2.95		-2.75 ± 6.56		53.4 ± 39.8		15.3 ± 5.6	
Body mass index (BMI)		0.45		0.73		0.67		0.52		0.38
Normal (BMI <25 kg/m^2)	6.19 ± 5.48		2.59 ± 2.49		-3.60 ± 6.41		56.2 ± 41.7		14.9 ± 5.9	
Overweight (25 kg/m^2≤ BMI <30 kg/m^2)	5.95 ± 5.24		3.05 ± 3.55		-2.91 ± 7.02		56.4 ± 35.9		15.2 ± 4.9	
Obese (BMI ≥30 kg/m^2)	4.67 ± 3.40		2.76 ± 2.07		-1.91 ± 4.36		47.0 ± 24.1		16.4 ± 6.7	
Sleep duration (hours/day)		0.10		0.64		0.16		0.04		0.96
≤5	4.97 ± 3.96		2.65 ± 2.09		-2.32 ± 4.83		48.4 ± 34.1		15.2 ± 6.2	
>5	6.15 ± 5.42		2.81 ± 3.02		-3.34 ± 6.74		56.7 ± 38.6		15.2 ± 5.9	
Wake time		0.98		0.30		0.75		0.02		0.99
Morning (4:00AM≤hh:hh<12:00AM)	6.20 ± 5.93		2.69 ± 2.65		-3.52 ± 6.96		62.0 ± 44.3		15.2 ± 5.9	
Afternoon (12:00AM≤hh:hh<8:00PM)	5.33 ± 3.50		2.44 ± 3.01		-2.89 ± 4.98		51.0 ± 29.4		15.3 ± 5.7	
Evening (8:00PM≤hh:hh<4:00AM)	5.63 ± 4.34		3.05 ± 3.10		-2.58 ± 5.96		45.0 ± 24.8		15.2 ± 5.5	
Work shift		0.05		0.08		0.56		0.04		0.38
Day	6.61 ± 5.34		2.38 ± 2.24		-4.22 ± 6.17		60.0 ± 36.3		15.6 ± 6.2	
Night	5.51 ± 5.03		2.99 ± 3.12		-2.52 ± 6.45		52.2 ± 38.4		15.0 ± 5.4	

Work sector (fatigue)		0.61	0.76	0.86	0.82	0.68
Less fatiguing	6.28 ± 6.16	2.70 ± 2.77	-3.59 ± 7.32	54.0 ± 38.3	15.4 ± 6.0	
More fatiguing	5.73 ± 4.67	2.80 ± 2.89	-2.93 ± 5.96	55.4 ± 37.6	15.1 ± 5.6	

Table 3. Conditional logistic regression results for the associations between metabolic syndrome (MetS) and salivary cortisol parameters and perceived stress scale with in female shift workers with MetS (cases) and without MetS (controls) (n=250), Southern Brazil, 2011

	Cases	Controls	Aged-adjusted (matched)		Multivariable-adjusted	
Cortisol Parameters/ PSS-10	**n (%)**	**n (%)**	**OR (95% CI)**	***p*-trend**	**OR (95% CI)**	***p*-trend**
Cortisol level immediately after waking (nmol/l)				0.29		0.69
Top tertile (≥6.68)	17 (34.0)	72 (36.0)	1.00 (ref. cat.)		1.00 (ref. cat.)	
Mid tertile (2.76-6.67)	12 (24.0)	67 (33.5)	0.76 (0.33, 1.76)		0.44 (0.17, 1.14)	
Bottom tertile (≤2.75)	21 (42.0)	61 (30.5)	1.43 (0.69, 2.96)		1.03 (0.46, 2.33)	
Cortisol level after work (nmol/l)				0.70		0.82
Bottom tertile (≤1.29)	13 (26.0)	67 (33.5)	1.00 (ref. cat.)		1.00 (ref. cat.)	
Mid tertile (1.30-2.60)	21 (42.0)	64 (32.0)	1.75 (0.79, 3.88)		1.73 (0.75, 4.02)	
Top tertile (2.61)	16 (32.0)	69 (34.5)	1.21 (0.55, 2.68)		1.14 (0.49, 2.66)	
Change in cortisol level (difference)				0.94		0.64
Bottom tertile (decline)	18 (36.0)	69 (34.5)	1.00 (ref. cat.)		1.00 (ref. cat.)	
Mid tertile (stable)	14 (28.0)	60 (30.0)	0.89 (0.41, 1.95)		0.79 (0.35, 1.79)	
Top tertile (rise)	18 (36.0)	71 (35.5)	0.97 (0.47, 1.99)		1.23 (0.56, 2.67)	
Area Under Curve - AUC (nmol/l/h)				0.87		0.63
Bottom tertile (≤31.9)	17 (34.0)	64 (32.0)	1.00 (ref. cat.)		1.00 (ref. cat.)	
Mid tertile (32.0-59.4)	16 (32.0)	68 (34.0)	0.89 (0.41, 1.90)		0.97 (0.44, 2.16)	
Top tertile (≥59.5)	17 (34.0)	68 (34.0)	0.94 (0.44, 2.01)		1.25 (0.54, 2.86)	
PSS-10 score (0-40)				0.51		0.29
Bottom tertile (≤13)	21 (42.0)	71 (35.5)	1.00 (ref. cat.)		1.00 (ref. cat.)	
Mid tertile (14-18)	16 (32.0)	73 (36.5)	0.75 (0.37, 1.54)		0.72 (0.34, 1.51)	
Top tertile (≥19)	13 (26.0)	56 (28.0)	0.80 (0.38, 1.69)		0.67 (0.30, 1.50)	


14
12
10
8
6
4
2
0
Cortisol level (nmol/l)
*
§
§
§
Morning
Afternoon
Evening
Morning
Afternoon
Evening
Waking
After work
Cases
Controls

Legends of tables

Table 1.

*P: values of t test for continuous variables and Fisher's exact test for categorical variables (heterogeneity of proportions); SD: standard deviation; HDL-C: high-density lipoprotein cholesterol; BP: blood pressure; PSS-10: Perceived Stress Scale with 10 items; ABEP: Brazilian Association of Research Companies - Economic Classification Criterion.

Table 2.

Values are presented as mean ± standard deviation. P: values of t test or one-way ANOVA test. PSS-10: Perceived Stress Scale with 10 items.

Table 3.

OR: Odds Ratio; PSS-10 perceived stress scale with 10 items: 95% CI: 95% Confidence Interval; P-trend: values for linear trend test from conditional logistic regression for age-matched case-control groups; Multivariable: adjusted for race, economic class, smoking status, physical activity, body mass index, sleep duration, time of awakening, and work shift.

Figure 1.

Comparative bar chart presenting the cortisol levels in saliva immediately after waking and one upon returning home from work by female shift workers with MetS (cases) and without MetS (controls) and stratified by their wake time: Morning (4:00AM≤hh:hh<12:00AM); Afternoon (12:00AM≤hh:hh<8:00PM); Evening (8:00PM≤hh:hh<4:00AM). Data are expressed as mean and error bars represent positive standard deviations. Two-factor analysis of variance (ANOVA) with repeated measures on one factor (Cases/Controls) was applied using log transformed data, followed by Bonferroni post hoc multiple comparison tests: * indicates significant difference ($p<0.05$) for Morning-Afternoon-Evening Cases vs. Controls (non-repeated measure); § indicates significant difference ($p<0.05$) within Cases and Controls (repeated measure).