# Seasonal differences in rhythmicity of salivary cortisol in healthy adults

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**Running title:** Seasonal rhythm of cortisol

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## ABSTRACT

The existence of seasonal changes in secretion of stress hormones and inflammatory mediators by humans is not certain. Here, we aimed to determine whether concentrations of cortisol and interleukin-6 (IL-6) displayed seasonal rhythmicity. The study was performed in Poznan, Poland (52°N, 16°E) in 7 healthy female volunteers (aged 22.6 ± 0.8 years). Samples of whole mixed unstimulated saliva were collected in winter (February) and summer (June) at 2-hour intervals over a 24-hour period, and analysed for cortisol and IL-6 by immunoassays. At each season the subjects answered questionnaires related to their sleeping habits, food intake, physical activity, and perceived seasonality. It turned out that salivary concentrations of cortisol followed a daily rhythm both in winter and summer, as determined by a cosine analysis. However, compared with the winter season, a midline estimating statistic of rhythm in the summer was significantly higher. Moreover, the rhythm acrophase occurred approximately 4 hours later in the summer than in the winter, while the amplitudes did not differ. These fluctuations did not correspond to sleeping habits, food and fluid intake, physical exercise, and the self-assessed chronotype. However, the individuals with higher scores in the seasonal affective disorder scale showed a tendency towards lower relative cortisol amplitude in the summer. In contrast to cortisol, salivary IL-6 concentration did not display daily rhythmicity and its concentrations did not differ significantly between the seasons. In conclusion, in the summer, cortisol level in saliva is elevated and its circadian pattern of secretion is shifted. The causes for these alterations do not seem to be related to lifestyle and thus remain to be established.

## INTRODUCTION

The biological significance of seasonal and daily environmental rhythms has long been appreciated (34). It is best seen in seasonal animals and hibernators, which adjust their physiology both in preparation for and in response to changing demands of the environment (9). Humans, like other organisms, have evolved an internal timing system consisting of self-sustained oscillators that can be reset by various synchronizers. The daily timing system comprises several components. The suprachiasmatic nucleus (SCN, a “body clock”) constitutes the endogenous element of an observed daily rhythm, superimposed upon which are direct effects of the environment and individual’s lifestyle, such as being inactive or not eating when asleep. These effects contribute the exogenous component to the observed daily rhythm.

Whilst the function of light-dark cycles for the adjustment (entrainment) of the body clock to the periodic environment is well recognized (33), the role of seasonal rhythms is less clear, as the existence and location of an endogenous “circannual” oscillator in humans is uncertain. Nevertheless, the seasonal rhythms do exist and may be linked to exogenous factors. These include seasonal differences in food intake, physical activity, temperature and the duration of natural light (12). In humans living in modern societies, the impact of seasonality has somewhat diminished after the introduction of artificial lighting, heating and air-conditioning systems, which reduce exposure to fluctuations in ambient temperature and light. However, ignoring seasonal changes may increase the risk of a mismatch between artificial indoor conditions and endogenous rhythms. Such misalignments may lead to alterations in metabolism and thermoregulation, which promote obesity (19; 46).

Cortisol is a life-sustaining hormone essential to the maintenance of homeostasis. There is a number of reports on seasonal rhythmicity in cortisol secretion, however, their results are inconsistent. Cortisol secretion follows a well-recognised circadian pattern (18; 28), and although this aspect of cortisol secretion is broadly studied, it is still far away from understanding. It is believed that circadian rhythm of cortisol secretion provides a link between body and mind (14; 47). Moreover, Kalafatakis et al. have recently demonstrated that cortisol secretion may exhibit ultradian rhythmicity that is critical for regulating behavioural and cognitive responses in humans (21).

However, little is known about seasonal variations of cortisol (22). This is partly because such studies demand detailed sampling over a 24 h-period performed under the same conditions at least two times per year, which is necessary to establish if there are any associations between daily and seasonal rhythms. In fact, there are conflicting reports on whether seasonality affects the daily rhythm of serum cortisol. Some studies showed seasonal variability in cortisol with the lowest concentrations recorded in summer (15), while others detected no seasonal rhythms in either serum cortisol or IL-6 (2; 22).

Interleukin-6 (IL-6) is a key mediator of inflammation (29; 36), whose secretion may also display daily fluctuations (16). However, the seasonal aspect of its secretions is poorly recognized. For the interpretation of potential seasonal variations in both cortisol and IL-6, it is important to determine whether the certain aspects of lifestyle, such as sleep, physical activity or the frequency of meals, associate with these parameters. In this respect it has been demonstrated (4) that short photoperiods contribute to seasonal affective disorder (SAD), which is associated with increased appetite, sleep disturbances and hypercorticolism (20).

Here, we aimed to verify a hypothesis that changes in cortisol and IL-6 occur seasonally in response to different photoperiods and are associated with certain lifestyle features.

## METHODS

### Test subjects and study design

Seven healthy female volunteers were studied. They were recruited from among the students of Poznan University of Medical Sciences. Their detailed demographic and anthropometric characteristics is given in Table 1. None of the subjects had a history of severe disease including cancer, autoimmune or endocrine disorders, and had no symptoms of infection or pollen allergy. As it is known that the amplitude of cortisol rhythm can be blunted in the luteal phase of the menstrual cycle (5), the subjects were assessed in the follicular phase. Moreover, all participants reported regular menstrual cycles and were not taking any interfering medication (including contraceptives). None of the subjects smoked. The study was carried out in Poznań (west-central Poland; 52°N latitude and 16°E longitude). The volunteers were examined two times over the year, in winter (February) and in summer (June). On each occasion the subjects were asked to choose 4 consecutive test days reflecting as closely as possible their daily routines and sleep-wake schedules. During these days they were asked to record the details of their physical activity, diet and sleep. The sleep was allowed, and its duration and quality of sleep were recorded by self-reports. The exact timing of meals during test days was not set, but the frequency and the composition of meals was recorded. In addition, on one day, they were asked to collect samples of saliva at designated time intervals (see below for details).

As actual duration of ambient light during test days has been implicated in shifting phases of hormonal rhythms (10; 38), we asked the National Institute of Meteorology and Water Management for the relevant data (Table 2). The study protocol followed the guidelines of the Journal for Human Biological Rhythm Research (32; 42) and was approved by the Ethics Committee of the Poznań University of Medical Sciences. All participants gave their informed consent.

## Saliva collection

Unstimulated whole mixed saliva was collected using Salivette® swabs (Sarstedt, Nümbrecht, Germany), as per manufacturer's instructions. Samples were collected over a 24-hour period at 2-hour intervals starting at 20:00 h. Following centrifugation, the samples of clear saliva were stored at -80°C until assayed in batch (35). The concentrations of cortisol and IL-6 were measured with specific immunoassays from DiaMetra (Segrate, Italy) and Diaclone (Besancon, France), respectively. The sensitivity of the assays was 0.12 ng/ml for cortisol and 2 pg/ml for IL-6. The assays were performed according to the manufacturers' instructions.

## Questionnaires

The participants were asked to answer a 25-question survey on their habits regarding sleep, food intake, physical activity, and seasonality. The questionnaire included questions on 1) demographics, height and weight measures, and chronotype; 2) sleep patterns and 3) dietary habits and physical activity. In addition, the participant were asked to answer the Seasonal Pattern Assessment Questionnaire, (SPAQ) (37).

## Statistical analysis

The data are presented as means ± standard deviations, or medians and ranges, as appropriate. Normality of the data distribution was tested with the Shapiro-Wilk's test. The paired data  were compared either with the paired t-test, ANOVA (for normally distributed data), or with the Wilcoxon test. Unpaired non-parametric data were analysed with either the Friedman test or the Dunn's Multiple Comparisons test. The relationship between variables was analyzed with Spearman’s rank correlation coefficient. All results were considered significant at $p<0.05$. Statistical analyses were performed with STATISTICA 13.0 (StatSoft Inc., Tulsa, USA) or InStat 3.06 (GraphPad Software Inc., La Jolla, USA). The daily rhythm was assessed by a single cosine test using Time Series Analysis Cosinor 6.3 software (Expert Soft Technologies, Laboratory of Applied Statistics and BioMedical Computing, Richelieu, France) and by MemCalc/Win (GMS, Tokyo, Japan).

## RESULTS

### Salivary cortisol and IL-6

Salivary concentrations of cortisol measured over a 24-hour period displayed conspicuous fluctuations. The cosine analysis revealed that these fluctuations exhibited a daily rhythm that could be seen both in the winter and in the summer (Figure 1). The characteristics of these rhythms are given in Table 3. Notably, midline estimating statistic of the rhythm (MESOR) in winter was significantly lower compared with that in summer ($p<0.02$). While, the rhythms had very similar amplitudes, they did differ in their acrophases. The acrophase of the cortisol rhythm in winter occurred almost 4 hours earlier than that in the summer ($p<0.02$). Accordingly, the greatest relative increase in salivary cortisol (delta (Δ) cortisol) was observed between 4 and 6 am in the winter and between 6 and 8 am in the summer.

In contrast to cortisol, the salivary concentrations of IL-6 did not follow a pattern to which a cosine wave could be fitted with a significant amplitude. This was the case for the IL-6 levels recorded both in the summer and in the winter. There was also no significant difference between the seasons in mean IL-6 concentrations at various times during the day (Fig. 2). To ensure that the potential differences in this respect were not obscured by inter-individual variability, the levels of IL-6 were analysed as percentages of individual 24-h means (not shown). This analysis also revealed no consistent differences in IL-6 between the seasons at any time point.

### Seasonality score

The mean seasonality score, as assessed with the SPAQ questionnaire, was 11.0 ± 0.6. According to the criteria by Kasper et al. (23), SAD was defined by scores ≥11.0. Five out of 7 subjects (71%) scored ≥11.0, and thus were classified as having SAD. The individual values for key parameters of the cortisol rhythm in SAD and non-SAD subjects are listed in Table 4 and Spearman coefficients for correlation between SAD score and the relative cortisol amplitude (the amplitude/mesor ratio) are shown in Table 5. Although there appeared to be a strong inverse correlation (r=-0.66) seen in the summer, it did not reach formal significance.

### Sleep, activity, food intake

To determine whether seasonal changes in salivary cortisol correlated in any way with food intake, physical activity and sleep patterns, the appropriate questionnaires were analysed (Table 6). These revealed no significant differences between the seasons in terms of sleeping and eating patterns. The duration of daily physical exercise tended to increase during the summer by about 40 minutes (p=0.08), but other activities did not change significantly.

## DISCUSSION

By applying a cosinor analysis, we wished to determine whether salivary cortisol and IL-6 exhibited circadian and seasonal rhythmicity. Our main observation is that salivary levels of cortisol, but not of IL-6, differ between the seasons and are higher in summer than in winter. The concentration of cortisol is typically the highest in the morning and gradually decreases throughout the day. Such a pattern was observed in both seasons. However, the MESOR for cortisol was significantly higher in summer than in winter. Moreover, the acrophase of the cortisol rhythm differed significantly between the seasons. In the summer, the acrophase occurred at approximately 13:13 h, while in the winter at 09:23 h.

The release of corticosteroids is stimulated by ACTH. The animal studies have demonstrated that ACTH and corticosterone are positively linked and exhibit parallel rhythms (30). Otsuka et al. have shown that photoperiod regulates the rhythmicity of plasma corticosterone in rats by modulating the sensitivity of the adrenals to ACTH (31). Our earlier study in humans detected seasonal changes in plasma ACTH with its levels being approximately 40% higher in the summer (22). While, the exact reason for such an increase in ACTH is unclear, it could explain the summer rise in salivary cortisol observed in the present study. Leproult et al. have shown that the effect of bright light on the hypothalamic-pituitary-adrenal (HPA) axis depends on the time of the day, being the greatest in the morning (25). In this respect, it has been demonstrated that humans exposed to long nights exhibit a slower increase in cortisol levels compared with individuals experiencing short nights (44). Vondrasova et al. observed that the morning rise in plasma cortisol occurred at about 03:00 h in summer and at approximately 05:00 h in winter (43). These findings are in contrast to our observations. We found that the greatest incremental increase in salivary cortisol occurred between 06:00 and 08:00 in the summer compared to 04:00-06:00 in the winter. The reason for such a discrepancy is unclear. It has been hypothesised that exposure to artificial light after dark suppresses some physiological responses including the rise in cortisol (45). It has been observed that peak hormonal secretion shifts to the morning hours when the activity continues late into the night (3). It appears, however, that the activity of our test subjects in the summer was no longer than in the winter. Moreover, we found no difference in the salivary cortisol rhythm between subjects of various chronotypes. This is consistent with a previous study by Toda et al. (2013), who found no differences in salivary cortisol between the “morning” and “evening” chronotypes of 108 male university students (40). Moreover, as the lifestyle of our subjects did not differ considerably between the seasons, these also are unlikely to account for the differences in cortisol rhythms observed. It remains to be determined whether a tendency toward greater physical activity in the summer could have contributed to a slight increase in daytime cortisol.

IL-6 is a strong activator of the HPA axis (27). However, we did not observe that changes in cortisol rhythmicity between the seasons correlated with salivary IL-6. The popular view holds it that increasingly hot summer seasons impose additional stress on humans and this may lead to increased secretion of stress hormones, including cortisol (1; 11). It has been suggested that the magnitude of this increase depends on the hydration status (13). Indeed, it has recently been demonstrated that acute exercise in the heat can increase both IL-6 and cortisol when fluid intake is restricted (6).

In contrast to cortisol, salivary IL-6 in our test subjects did not display circadian rhythmicity and did not differ between summer and winter. This is unlike our previous study (22), which detected seasonal changes in serum IL-6. This apparent discrepancy could be related to different sampling frequency (every 2 hours over 24 hours vs. once a day) and the use of different test medium (saliva vs. serum) . In this respect, the diagnostic potential of saliva is increasingly used in biomedical research (26). Saliva is well suited for studies in chronobiology as its multiple samples can easily and frequently be collected in a non-invasive manner. With regard to salivary IL-6, our observations need to be confirmed by independent investigations, since no studies so far examined salivary IL-6 levels across the seasons using a repeated design. With respect to cortisol, it is recognized that salivary cortisol represents the biologically active hormone (24) and its levels in saliva follow a similar daily rhythm as that in serum (8).

Five out of seven volunteers examined in the present study reported seasonal mood changes. The analysis for correlation between SAD score and cortisol amplitude suggested an inverse correspondence between the two, with a stronger pattern in the summer. While this association came out as insignificant in the analysis, it might have been related to a small sample size. Thorn et al showed similar cortisol profiles in SAD and non-SAD individuals both in summer and winter. However, they found that in winter SAD individuals showed an attenuated cortisol response to awakening (39). This observation, together with our findings, may indicate that SAD is associated with seasonal changes in cortisol rhythmicity .

Whilst our findings confirm earlier reports that normal subjects do exhibit seasonal variations in mood and behaviour (37), they do not provide support for the view that seasonal changes could be related to alterations in sleep hygiene (41). We found no seasonal differences in sleeping patterns either during working days or during weekends. This is in contrast to the observations by Hashizaki et al. (17), who reported that the sleep onset time did not show clear seasonal variations, but the sleep rise time changed with seasons occurring later in winter than in summer. The difference between the studies can be at least partly related to different latitudes of where the studies were conducted, i.e. lower latitudes in Japan vs. higher latitudes in Poland.

Seasonal changes may affect availability of certain foods, individuals' feeding habits and outdoor activity – with picnics, for example, being more common in the summer (33). It has been observed that the amount of food eaten shows seasonal variations, with increased meal sizes and increased total calorie intake in autumn (7). In our test subjects we observed a tendency towards prolonged exercise in summer, but no seasonal differences in the number of meals eaten and in the sleep habits.

In conclusion, our study demonstrates that salivary concentrations of cortisol differ significantly between the seasons in terms of both average levels and daily rhythmicity. The exact causes for elevated and shifted cortisol secretion in the summer remain to be determined. However, they do not seem to be overtly related to seasonal changes in eating or sleeping patterns.

Although being a pilot study, our investigation was performed on only a few volunteer participants, a very dense and strict sampling procedure throughout the experiments allowed us to detected with a high degree of certainty the presence of seasonal differences in circadian cortisol rhythms. Obviously, our observations need to be verified in future better-powered studies. Nevertheless, this line of investigation is of clinical importance as seasonal changes in cortisol secretion are increasingly implicated in psychological and psychiatric disorders (35).

## DECLARATIONS

The data were presented in the abstract form at the American Physiological Society Experimental Biology annual meeting (San Diego, CA, USA 2018).

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Ethics Committee of the Poznan University of Medical Sciences and was performed in accordance with the Helsinki Declaration. All participants gave written informed consent before entering the study.

## CONSENT FOR PUBLICATION

All authors have read the manuscript and have agreed to submit it in its current form for consideration for publication.

## AVAILABILITY OF DATA AND MATERIAL

The data supporting the conclusions of this article are included within the manuscript. The dataset is available from the corresponding author on request.

## COMPETING INTERESTS

All authors declare no conflict of interest.

## AUTHORS' CONTRIBUTIONS

Study concept and design: DK; data acquisition: DK, DS, RR; data analysis and interpretation: DK, DS; MS; drafting the manuscript: DK; MS; critical revision of the manuscript for important intellectual content: JW, AB, MS; statistical analysis: MR; administrative and technical support: RR; study supervision: JW. All authors read and approved the final manuscript.

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## REFERENCES

1. **Abell JG, Stalder T, Ferrie JE, Shipley MJ, Kirschbaum C, Kivimaki M and Kumari M**. Assessing cortisol from hair samples in a large observational cohort: The Whitehall II study. *Psychoneuroendocrinology* 73: 148-156, 2016
2. **Adamsson M, Laike T and Morita T**. Annual variation in daily light exposure and circadian change of melatonin and cortisol concentrations at a northern latitude with large seasonal differences in photoperiod length. *J Physiol Anthropol* 19;36: 6-0103, 2016.
3. **Arendt J**. Importance and relevance of melatonin to human biological rhythms. *J Neuroendocrinol* 15: 427-431, 2003.
4. **Arendt J and Middleton B**. Human seasonal and circadian studies in Antarctica (Halley, 75 degrees S). *Gen Comp Endocrinol* 258: 250-258, 2018.
5. **Baker FC and Driver HS**. Circadian rhythms, sleep, and the menstrual cycle. *Sleep Med* 8: 613-622, 2007.
6. **Costello JT, Rendell RA, Furber M, Massey HC, Tipton MJ, Young JS and Corbett J**. Effects of acute or chronic heat exposure, exercise and dehydration on plasma cortisol, IL-6 and CRP levels in trained males. *Cytokine* 110: 277-283, 2018.
7. **De Castro JM**. Seasonal rhythms of human nutrient intake and meal pattern. *Physiol Behav* 50: 243-248, 1991.
8. **Dorn LD, Lucke JF, Loucks TL and Berga SL**. Salivary cortisol reflects serum cortisol: analysis of circadian profiles. *Ann Clin Biochem* 44: 281-284, 2007.
9. **Ebling FJ and Barrett P**. The regulation of seasonal changes in food intake and body weight. *J Neuroendocrinol* 20: 827-833, 2008.

10. **Figueiro MG and Rea MS**. Evening daylight may cause adolescents to sleep less in spring than in winter. *Chronobiol Int* 27: 1242-1258, 2010.
11. **Fischer S, Duncko R, Hatch SL, Papadopoulos A, Goodwin L, Frissa S, Hotopf M and Cleare AJ**. Sociodemographic, lifestyle, and psychosocial determinants of hair cortisol in a South London community sample. *Psychoneuroendocrinology* 76: 144-153, 2017.
12. **Foster RG and Roenneberg T**. Human responses to the geophysical daily, annual and lunar cycles. *Curr Biol* 18: R784-R794, 2008.
13. **Francesconi RP, Sawka MN, Pandolf KB, Hubbard RW, Young AJ and Muza S**. Plasma hormonal responses at graded hypohydration levels during exercise-heat stress. *J Appl Physiol (1985 )* 59: 1855-1860, 1985.
14. **Gonnissen HK, Rutters F, Mazuy C, Martens EA, Adam TC and Westerterp-Plantenga MS**. Effect of a phase advance and phase delay of the 24-h cycle on energy metabolism, appetite, and related hormones. *Am J Clin Nutr* 96: 689-697, 2012.
15. **Hadlow NC, Brown S, Wardrop R and Henley D**. The effects of season, daylight saving and time of sunrise on serum cortisol in a large population. *Chronobiol Int* 31: 243-251, 2014.
16. **Hartman J and Frishman WH**. Inflammation and atherosclerosis: a review of the role of interleukin-6 in the development of atherosclerosis and the potential for targeted drug therapy. *Cardiol Rev* 22:147-151, 2014.
17. **Hashizaki M, Nakajima H, Shiga T, Tsutsumi M and Kume K**. A longitudinal large-scale objective sleep data analysis revealed a seasonal sleep variation in the Japanese population. *Chronobiol Int* 35: 933-945, 2018.
18. **Haus E**. Chronobiology in the endocrine system. *Adv Drug Deliv Rev* 59: 985-1014, 2007.

19. **Johnson F, Mavrogianni A, Ucci M, Vidal-Puig A and Wardle J**. Could increased time spent in a thermal comfort zone contribute to population increases in obesity? *Obes Rev* 12: 543-551, 2011.

20. **Joseph-Vanderpool JR, Rosenthal NE, Chrousos GP, Wehr TA, Skwerer R, Kasper S and Gold PW**. Abnormal pituitary-adrenal responses to corticotropin-releasing hormone in patients with seasonal affective disorder: clinical and pathophysiological implications. *J Clin Endocrinol Metab* 72: 1382-1387, 1991.

21. **Kalafatakis K, Russell GM, Harmer CJ, Munafo MR, Marchant N, Wilson A, Brooks JC, Durant C, Thakrar J, Murphy P, Thai NJ and Lightman SL**. Ultradian rhythmicity of plasma cortisol is necessary for normal emotional and cognitive responses in man. *Proc Natl Acad Sci U S A* 115: E4091-E4100, 2018.

22. **Kanikowska D, Sugenoya J, Sato M, Shimizu Y, Inukai Y, Nishimura N and Iwase S**. Seasonal variation in blood concentrations of interleukin-6, adrenocorticotrophic hormone, metabolites of catecholamine and cortisol in healthy volunteers. *Int J Biometeorol* 53: 479-485, 2009.

23. **Kasper S, Wehr TA, Bartko JJ, Gaist PA and Rosenthal NE**. Epidemiological findings of seasonal changes in mood and behavior. A telephone survey of Montgomery County, Maryland. *Arch Gen Psychiatry* 46: 823-833, 1989.

24. **Kirschbaum C and Hellhammer DH**. Salivary cortisol in psychoneuroendocrine research: recent developments and applications. *Psychoneuroendocrinology* 19: 313-333, 1994.

25. **Leproult R, Colecchia EF, L'Hermite-Baleriaux M and Van CE**. Transition from dim to bright light in the morning induces an immediate elevation of cortisol levels. *J Clin Endocrinol Metab* 86: 151-157, 2001.

26. **Liu J and Duan Y**. Saliva: a potential media for disease diagnostics and monitoring. *Oral Oncol* 48: 569-577, 2012.

27. **Mastorakos G, Weber JS, Magiakou MA, Gunn H and Chrousos GP**. Hypothalamic-pituitary-adrenal axis activation and stimulation of systemic vasopressin secretion by recombinant interleukin-6 in humans: potential implications for the syndrome of inappropriate vasopressin secretion. *J Clin Endocrinol Metab* 79: 934-939, 1994.

28. **Mavroudis PD, Corbett SA, Calvano SE and Androulakis IP**. Circadian characteristics of permissive and suppressive effects of cortisol and their role in homeostasis and the acute inflammatory response. *Math Biosci* 260:54-64, 2015.

29. **Mihara M, Hashizume M, Yoshida H, Suzuki M and Shiina M**. IL-6/IL-6 receptor system and its role in physiological and pathological conditions. *Clin Sci (Lond)* 122: 143-159, 2012.

30. **Oster H, Damerow S, Kiessling S, Jakubcakova V, Abraham D, Tian J, Hoffmann MW and Eichele G**. The circadian rhythm of glucocorticoids is regulated by a gating mechanism residing in the adrenal cortical clock. *Cell Metab* 4: 163-173, 2006.

31. **Otsuka T, Goto M, Kawai M, Togo Y, Sato K, Katoh K, Furuse M and Yasuo S**. Photoperiod regulates corticosterone rhythms by altered adrenal sensitivity via melatonin-independent mechanisms in Fischer 344 rats and C57BL/6J mice. *PLoS ONE* 7: e39090, 2012.

32. **Portaluppi F, Smolensky MH and Touitou Y**. Ethics and methods for biological rhythm research on animals and human beings. *Chronobiol Int* 27: 1911-1929, 2010.

33. **Reilly T and Peiser B.** Seasonal variations in health-related human physical activity. *Sports Med* 36: 473-485, 2006.

34. **Reinberg A.** The significance of biological rhythms in biometeorology. Biological rhythms and human biometeorology, with special reference to mortality rhythms and chronotoxicology. *Int J Biometeorol* 16 Suppl: 97-112, 1972.

35. **Riis JL, Out D, Dorn LD, Beal SJ, Denson LA, Pabst S, Jaedicke K and Granger DA.** Salivary cytokines in healthy adolescent girls: Intercorrelations, stability, and associations with serum cytokines, age, and pubertal stage. *Dev Psychobiol* 56: 797-811, 2014.

36. **Rincon M.** Interleukin-6: from an inflammatory marker to a target for inflammatory diseases. *Trends Immunol* 33: 571-577, 2012.

37. **Rosenthal NE, Rotter A, Jacobsen FM and Skwerer RG.** No mood-altering effects found after treatment of normal subjects with bright light in the morning. *Psychiatry Res* 22: 1-9, 1987.

38. **Stokkan KA and Reiter RJ.** Melatonin rhythms in Arctic urban residents. *J Pineal Res* 16: 33-36, 1994.

39. **Thorn L, Evans P, Cannon A, Hucklebridge F and Clow A.** Seasonal differences in the diurnal pattern of cortisol secretion in healthy participants and those with self-assessed seasonal affective disorder. *Psychoneuroendocrinology* 36: 816-823, 2011.

40. **Toda M, Kawai T, Takeo K, Rokutan K and Morimoto K.** Associations between chronotype and salivary endocrinological stress markers. *Endocr Res* 38: 1-7, 2013.

41. **Tonetti L, Fabbri M, Erbacci A, Martoni M and Natale V.** Association between seasonal affective disorder and subjective quality of the sleep/wake cycle in adolescents. *Psychiatry Res* 215: 624-627, 2014.

42. **Touitou Y, Smolensky MH and Portaluppi F.** Ethics, standards, and procedures of animal and human chronobiology research. *Chronobiol Int* 23: 1083-1096, 2006.

43. **Vondrasova D, Hajek I and Illnerova H.** Exposure to long summer days affects the human melatonin and cortisol rhythms. *Brain Res* 759: 166-170, 1997.

44. **Wehr TA.** Effect of seasonal changes in daylength on human neuroendocrine function. *Horm Res* 49: 118-124, 1998.

45. **Wehr TA, Giesen HA, Moul DE, Turner EH and Schwartz PJ.** Suppression of men's responses to seasonal changes in day length by modern artificial lighting. *Am J Physiol* 269: R173-R178, 1995.

46. **Wyse CA, Selman C, Page MM, Coogan AN and Hazlerigg DG.** Circadian desynchrony and metabolic dysfunction; did light pollution make us fat? *Med Hypotheses* 77: 1139-1144, 2011.

47. **Zeitzer JM, Nouriani B, Rissling MB, Sledge GW, Kaplan KA, Aasly L, Palesh O, Jo B, Neri E, Dhabhar FS and Spiegel D.** Aberrant nocturnal cortisol and disease progression in women with breast cancer. *Breast Cancer Res Treat* 158: 43-50, 2016.

## LEGENDS TO TABLES AND FIGURES

**Table 1.** Participant characteristics.

**Table 2**. The characteristics of some meteorological parameters in summer and in winter.

**Table 3.** The characteristics of daily cortisol rhythm in summer and in winter.

**Table 4.** The characteristics of seasonal cortisol rhythms in individuals with or without SAD.

**Table 5.** The correlation analysis for SAD score and cortisol relative amplitude.

**Table 6.** Features of sleep , activity and dietary habits in different seasons.

**Fig. 1.** Changes in salivary cortisol concentration in summer (**A**) and winter (**B**). The data are presented as means ± SD of values recorded in 7 individuals. The dashed curve represents the fitted cosine wave. The solid line represents the calculated MESOR. Zero value on the X axis corresponds to midnight.

**Fig. 2.** Changes in salivary interleukin 6 in summer (**A**) and winter (**B**). The data are presented as means ± SD of values recorded in 7 individuals. Zero value on the X axis corresponds to midnight.

**Table 1.**

**Participant characteristics (n=7).**

| | |
|---|---|
| Age, years | 22.6 ± 0.8 |
| BMI, kg/m$^2$ | 20.52 ± 0.16 |
| Normal BMI (18.5-24.9 kg/m$^2$, n (%) | 7 (100%) |
| Waist (cm) | 67.7 ± 3.1 |
| Knowledge of healthy lifestyle, n (%) | 7 (100%) |
| Global seasonal score, points<br>• seasonal affective disorder, n (%)<br>• winter seasonal pattern, n (%) | 11.0 ± 4.7<br>5 (71%)<br>5 (71%) |
| Chronotype, n (%)<br>• “LARK” (like to do things early in the day and relax in the evening)<br>• “OWL” (like to relax in the morning and do things in the evening)<br>• “NEITHER “ (like to spread out things to do throughout the day) | <br>2 (29%)<br>3 (43%)<br>2 (29%) |

**Table 2.**

**The characteristics of meteorological parameters at the test site in summer and winter.**

| Parameter (mean) | Summer | Winter |
|---|---|---|
| Sunshine duration, h | 7.8 | 2.9 |
| Ambient natural photoperiod, h | 16.5 | 8.3 |
| Solar radiation, J/cm$^2$ | 1869.0 | 567.1 |
| Solar radiation between 04:00 and 09:00 h, J/cm$^2$ | 60.0 | 4.8 |
| Solar radiation between 17:00 and 21:00 h, J/cm$^2$ | 49.1 | 4.0 |

**Table 3.**

**The characteristics of daily cortisol rhythm in summer and in winter.**

| Parameter (mean ± SD) | Summer | Winter | P value |
|---|---|---|---|
| MESOR, ng/ml | 3.8 ± 0.9 | 2.8 ± 0.5 | 0.02 |
| Amplitude, ng/ml | 1.7 ± 0.5 | 1.7 ± 0.6 | ns |
| Acrophase, clock time | 13:13 | 09:23 | 0.02 |

The daily rhythm is described by the MESOR, amplitude , and acrophase. The MESOR (midline estimating statistic of rhythm) is the mean of all values across the circadian rhythm. The amplitude is half the difference between the highest and the lowest points of the cosine function best fitting the data. The acrophase represents the time point when the circadian cycle reaches the peak value.

**Table 4.**

**The characteristics of seasonal cortisol rhythms in individuals with or without SAD.**

| Subject | Global seasonal score | Summer | | | Winter | | |
|---|---|---|---|---|---|---|---|
| | | Amplitude, ng/ml | MESOR, ng/ml | Acrophase, clock time | Amplitude, ng/ml | MESOR, ng/ml | Acrophase, clock time |
| 1 | 5.0 | 2.6 | 3.1 | 10:25 | 2.6 | 3.0 | 08:52 |
| 2 | 6.0 | 2.3 | 3.6 | 14:52 | 1.6 | 3.3 | 09:25 |
| 3 | 11.0 | 2.9 | 6.0 | 13:10 | 2.7 | 3.7 | 10:39 |
| 4 | 11.0 | 1.4 | 3.0 | 12:26 | 2.5 | 2.9 | 11:03 |
| 5 | 12.0 | 1.9 | 3.6 | 17:45 | 1.2 | 2.0 | 07:07 |
| 6 | 13.0 | 1.5 | 3.7 | 12:08 | 2.4 | 2.9 | 10:07 |
| 7 | 19.0 | 1.5 | 3.1 | 12:28 | 1.4 | 2.3 | 08:39 |

SAD, seasonal affective disorder is defined by global seasonal score ≥11.0

**Table 5.**

**The correlation analysis for SAD score and cortisol relative amplitude**

| Variables | Spearman correlation analysis | | | |
|---|---|---|---|---|
| | R coefficient | p-value | N | Significance |
| SAD score and A/M (cortisol relative amplitude) in summer | -0.59 | 0.112 | 7 | Insignificant |
| SAD score and A/M (cortisol relative amplitude) in winter | -0.25 | 0.582 | 7 | Insignificant |

**Table 6.**

**Features of sleep, activity and dietary habits in different seasons.**

| | Winter | Summer | Significance (P) |
|---|---|---|---|
| *Sleep* | | | |
| Retiring time, clock time | 00:50 | 00:55 | ns |
| Rising time, clock time | 08:22 | 08:17 | ns |
| Length of sleep, hours | 7.26± 0.64 | 7.17 ±1.22 | ns |
| Overall sleep quality, score* | 3.43 ± 0.62 | 3.29 ± 0.74 | ns |
| Refreshed after sleep, score* | 3.14 ± 1.23 | 3.00 ± 0.80 | ns |
| *Activity (hours spent per day)* | | | |
| Lying | 1.77 ± 1.23 | 2.04 ± 1.10 | ns |
| Sitting | 9.05 ± 3.22 | 9.34 ± 2.69 | ns |
| Walking | 2.71 ± 0.99 | 3.05 ± 1.78 | ns |
| Exercise | 0.29 ± 0.29 | 0.75 ± 0.56 | ns (p=0.08) |
| Standing | 2.14 ± 1.16 | 2.0 ± 1.28 | ns |
| *Meals, times/day ** Mean ± SD* | | | |
| • breakfast | 0.75 ± 0.41 | 0.68 ± 0.40 | ns |
| • elevenses | 0.71 ± 0.22 | 0.75 ± 0.25 | ns |
| • lunch | 1.0 ± 0.41 | 0.96 ± 0.34 | ns |
| • tea | 0.93 ± 0.28 | 0.86 ± 0.35 | ns |
| • dinner | 1.04 ± 0.27 | 1.14 ± 0.24 | ns |
| • supper | 0.29 ± 0.37 | 0.32 ± 0.24 | ns |

* Sleep quality was scored from 1 to 5 (very bad – very good)
** Frequency of particular food consumption was scored as follows: 0 = Nothing eaten; 1 = Snack/One course meal; 2 = Two-course meal; 3 = Three-course meal or more
ns- statistically non-significant

**Figure 1.**

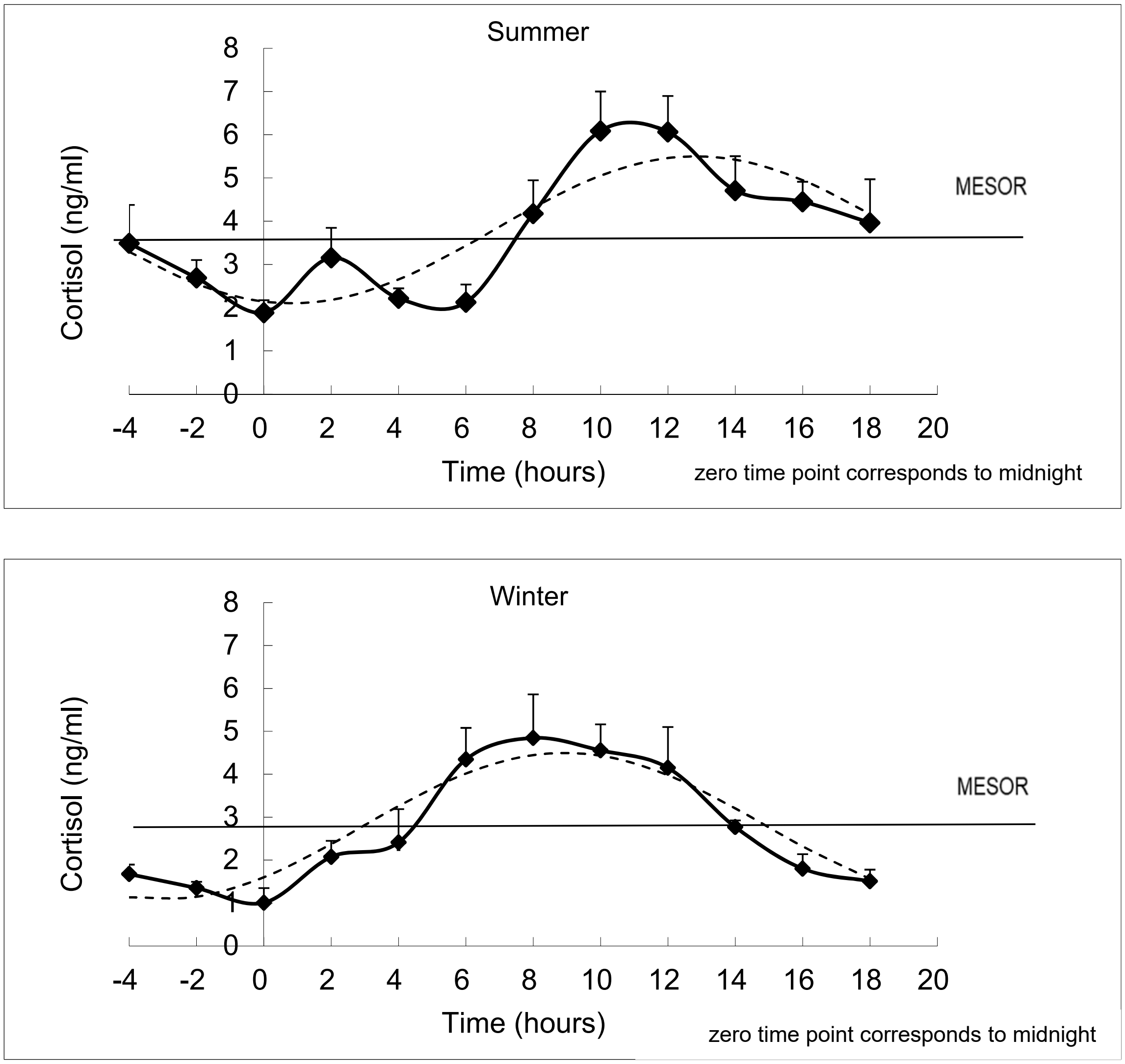
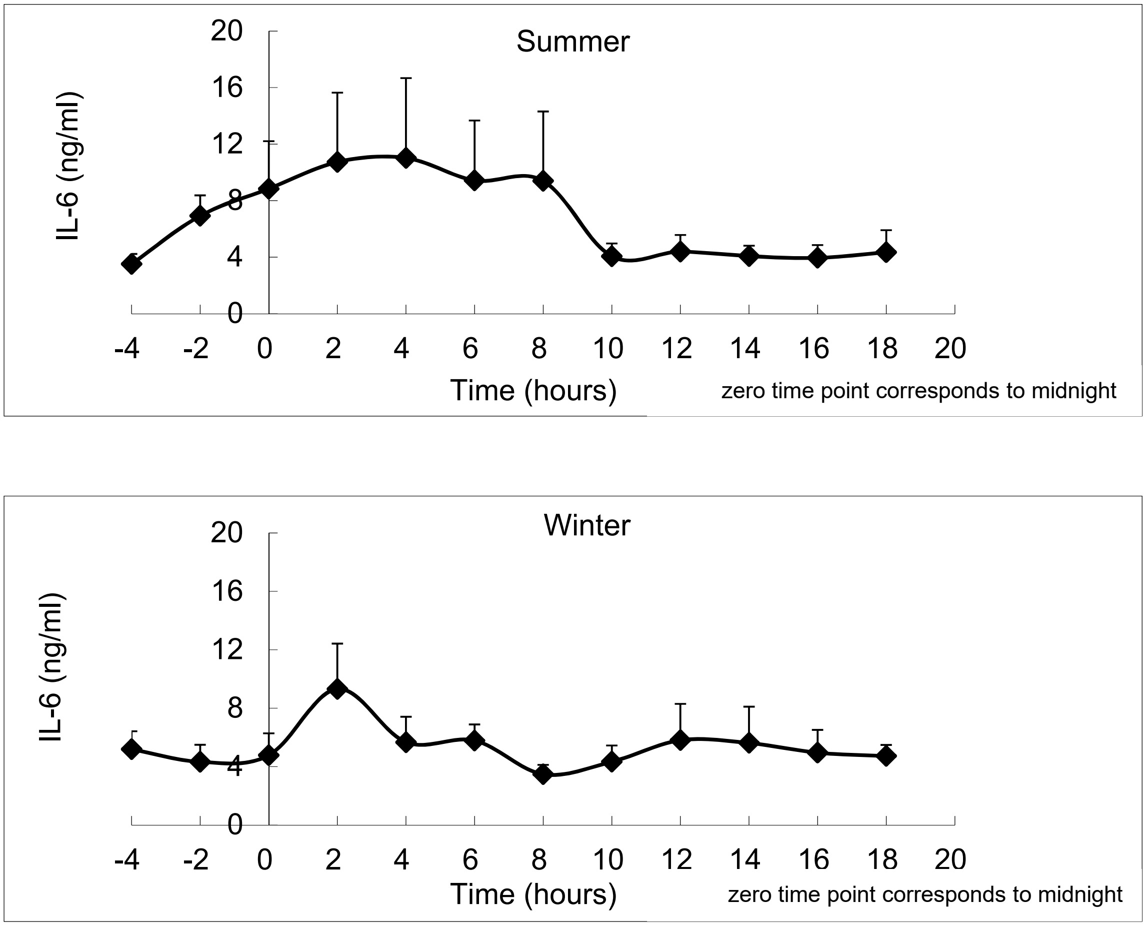

Summer
Cortisol (ng/ml)
0
1
2
3
4
5
6
7
8
MESOR
-4
-2
0
2
4
6
8
10
12
14
16
18
20
Time (hours)
zero time point corresponds to midnight
Winter
Cortisol (ng/ml)
0
1
2
3
4
5
6
7
8
MESOR
-4
-2
0
2
4
6
8
10
12
14
16
18
20
Time (hours)
zero time point corresponds to midnight

**Figure 2.**


Summer
IL-6 (ng/ml)
0
4
8
12
16
20
-4
-2
0
2
4
6
8
10
12
14
16
18
20
Time (hours)
zero time point corresponds to midnight
Winter
IL-6 (ng/ml)
0
4
8
12
16
20
-4
-2
0
2
4
6
8
10
12
14
16
18
20
Time (hours)
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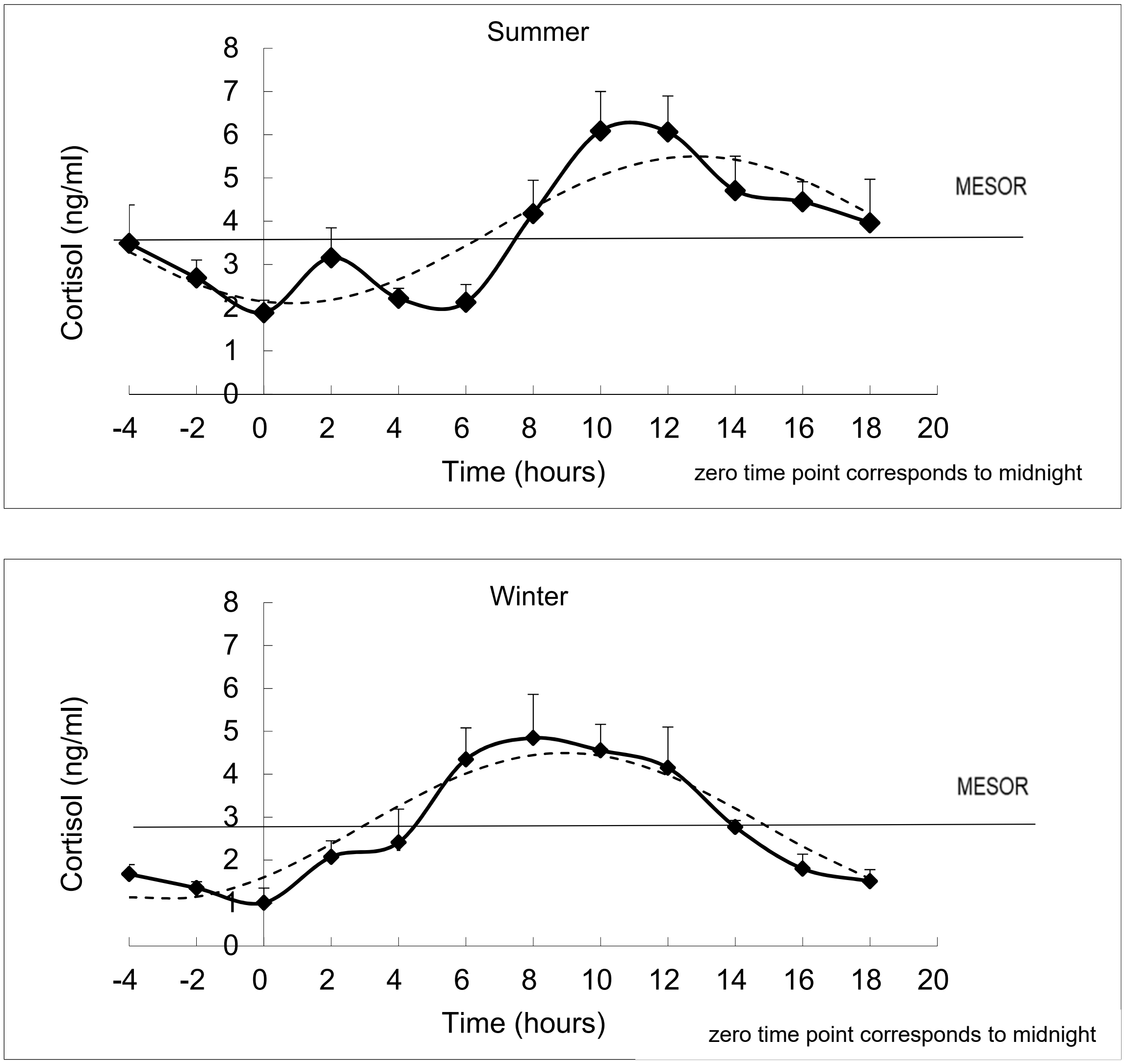
Summer
Cortisol (ng/ml)
0
1
2
3
4
5
6
7
8
-4
-2
0
2
4
6
8
10
12
14
16
18
20
Time (hours)
zero time point corresponds to midnight
MESOR
Winter
Cortisol (ng/ml)
0
1
2
3
4
5
6
7
8
-4
-2
0
2
4
6
8
10
12
14
16
18
20
Time (hours)
zero time point corresponds to midnight
MESOR

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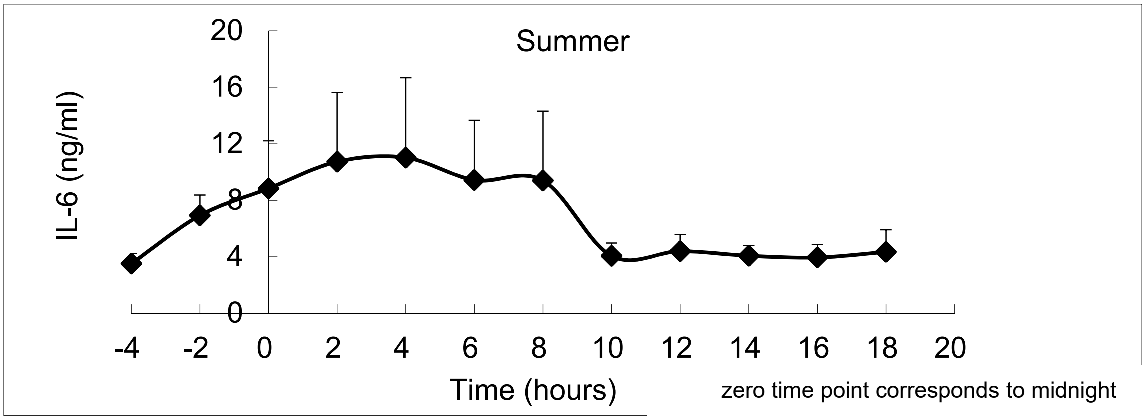
Summer
IL-6 (ng/ml)
20
16
12
8
4
0
-4
-2
0
2
4
6
8
10
12
14
16
18
20
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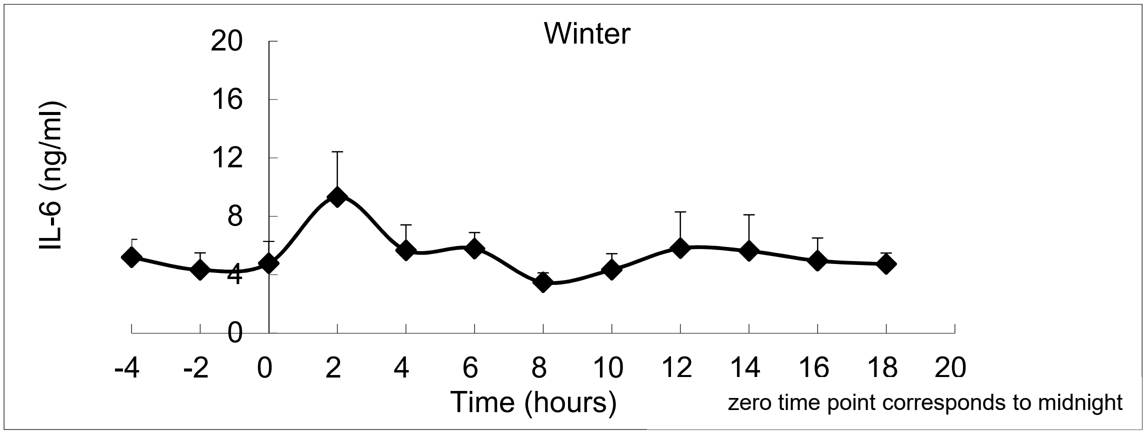
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20
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8
4
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-2
0
2
4
6
8
10
12
14
16
18
20
Time (hours)
zero time point corresponds to midnight

**Table 1.**

**Participant characteristics (n=7).**

| | |
|---|---|
| Age, years | 22.6 ± 0.8 |
| BMI, kg/m$^2$ | 20.52 ± 0.16 |
| Normal BMI (18.5-24.9 kg/m$^2$, n (%) | 7 (100%) |
| Waist (cm) | 67.7 ± 3.1 |
| Knowledge of healthy lifestyle, n (%) | 7 (100%) |
| Global seasonal score, points | 11.0 ± 4.7 |
| • seasonal affective disorder, n (%) | 5 (71%) |
| • winter seasonal pattern, n (%) | 5 (71%) |
| Chronotype, n (%) | |
| • "LARK" (like to do things early in the day and relax in the evening) | 2 (29%) |
| • "OWL" (like to relax in the morning and do things in the evening) | 3 (43%) |
| • "NEITHER " (like to spread out things to do throughout the day) | 2 (29%) |

**Table 2.**

**The characteristics of meteorological parameters at the test site in summer and winter.**

| Parameter (mean) | Summer | Winter |
|---|---|---|
| Sunshine duration, h | 7.8 | 2.9 |
| Ambient natural photoperiod, h | 16.5 | 8.3 |
| Solar radiation, J/cm$^2$ | 1869.0 | 567.1 |
| Solar radiation between 04:00 and 09:00 h, J/cm$^2$ | 60.0 | 4.8 |
| Solar radiation between 17:00 and 21:00 h, J/cm$^2$ | 49.1 | 4.0 |

**Table 3.**

**The characteristics of daily cortisol rhythm in summer and in winter.**

| Parameter (mean ± SD) | Summer | Winter | P value |
|---|---|---|---|
| MESOR, ng/ml | 3.8 ± 0.9 | 2.8 ± 0.5 | 0.02 |
| Amplitude, ng/ml | 1.7 ± 0.5 | 1.7 ± 0.6 | ns |
| Acrophase, clock time | 13:13 | 09:23 | 0.02 |

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