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Association between chronotype and body mass index: The role of C-reactive protein and the cortisol response to stress

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ABSTRACT

Background: : Chronotype influences several physiological systems, including the immune system and the hypothalamus-pituitary-adrenal (HPA)-axis. Previous research has shown that evening chronotype is associated with adverse metabolic health outcomes and obesity. However, the exact mechanisms underlying the observed differences in metabolic function between "morning" and "evening" types remain to be explored.
Objective: : To investigate the relationship of chronotype with inflammatory and neuroendocrine stress markers and to explore their mediating and moderating roles in the association between chronotype and body mass index (BMI).
Methods: : Twenty-eight healthy young adults (50% women), mean age 23.8 ± 3.3 (SD) years, underwent a standardized laboratory stress test (Trier Social Stress Test, TSST). Concentrations of plasma C-reactive protein (CRP) at baseline and of salivary cortisol before and after the onset of the stressor were analyzed. Heart rate was measured continuously. Chronotype was assessed with the Morningness-Eveningness Questionnaire (MEQ).
Results: : Lower MEQ scores (i.e. evening tendency) were associated with higher BMI ($r = -.40$, $p < .05$), elevated CRP concentrations ($r = -.42$, $p < .05$) and higher cortisol responses to acute stress ($r = -.53$, $p < .01$). The relationship between MEQ score and BMI was mediated by CRP concentrations ($b = -0.03$, CI 95%: –0.08 to –0.007, $p < .05$). In addition, we observed a moderating effect of the cortisol stress response on this mediated relationship ($b = 0.005$, CI 95%: 0.0002 to 0.01, $p < .05$), such that the mediated relationship was stronger in individuals with a higher cortisol response.
Conclusion: : Enhanced pro-inflammatory state and a higher cortisol response to stress may underlie the effect of evening chronotype on obesity risk and adverse metabolic health outcomes.

1. Introduction

Circadian rhythms and the circadian molecular clockwork are present in nearly all mammalian cells and play a major role in regulating daily rhythms of sleep/wake and various metabolic outputs, such as food intake behavior, peripheral tissue metabolism, and hormone secretions (Huang et al., 2011). The circadian system is controlled by the master circadian clock located in the suprachiasmatic nuclei (SCN) of the hypothalamus, which integrates input (e.g., daylight exposure) from the optic nerve and synchronizes circadian rhythms in the periphery. Circadian rhythms are both autonomous and self-sustainable but their phases are also continuously modified, or entrained, by external time cues, like daylight, time of meal consumption, diet composition and exercise in order to synchronize them with the external environment (Huang et al., 2011). The SCN maintains continuous communication with the peripheral clocks through a variety of neural and humoral signals, including glucocorticoid release by the hypothalamus–pituitary–adrenal (HPA) axis (Chung et al., 2011).

It is known that humans show large individual variability in entrainment characteristics, resulting in differences in timing of activity preference and preferred behavioral circadian rhythm, also described as "phase of entrainment" or "chronotype" in chronobiology (Horne and Ostberg, 1976; Huang et al., 2011). Morning or evening preference has proven to be a stable human behavioral trait (Horne and Ostberg, 1976) with a measurable and predictable impact on different physiological systems and psychological parameters like body temperature (Bailey

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and Heitkemper, 2001; Kerkhof and Van Dongen, 1996), blood pressure (Merikanto et al., 2013a; Uusitalo et al., 1988; Yu et al., 2015), catecholamine secretion (Akerstedt and Froberg, 1976), sleep patterns (Ishihara et al., 1987), subjective activation and arousal (Adan and Guardia, 1993; Akerstedt and Froberg, 1976) or lifestyle regularity (Monk et al., 2004). Evidence indicates that evening or late chronotype is associated with a greater degree of misalignment between internal timing and external conditions (Roenneberg et al., 2012; Wittmann et al., 2006), potentially leading towards disruptions in central and peripheral rhythms (Huang et al., 2011). Furthermore, individuals classified as the evening chronotype are at higher risk for developing poor health outcomes (Olds et al., 2011; Yu et al., 2015), including higher BMI (Arora and Taheri, 2014; Lucassen et al., 2013), the metabolic syndrome (Parsons et al., 2015; Yu et al., 2015), diabetes type 2 (Merikanto et al., 2013b; Yu et al., 2015), asthma (Merikanto et al., 2014) and depression (Merikanto et al., 2013a).

Chronotype influences HPA-axis function, including glucocorticoid release by the adrenal gland. Glucocorticoids are important regulators of lipid and glucose metabolism and alterations in HPA-axis function and have been associated with higher BMI and abdominal fat (Incollingo Rodriguez et al., 2015; Joseph and Golden, 2017). Chronotype acts upon the diurnal cortisol profile regulated by the HPA-axis. For example, several studies indicate significantly higher salivary cortisol levels in the first hour after awakening (Kudielka et al., 2007, 2006; Randler and Schaal, 2010) and an earlier occuring acrophase of the circadian rhythm of total cortisol (Bailey and Heitkemper, 2001) in morning compared to evening chronotypes. Also, females with the evening chronotype show significantly higher diurnal cortisol output on leisure days (Dockray and Steptoe, 2011) and in obese short-sleeping participants evening tendency is associated with higher morning plasma ACTH and urinary epinephrine concentrations (Lucassen et al., 2013). Furthermore, compared to morning types, evening types showed higher cortisol concentrations after high-intensity interval exercise performed early in the morning (Bonato et al., 2017) and displayed lower cortisol responses to a standardized psychosocial laboratory stressor (Marvel-Coen et al., 2018). Also time of day of exposure to a psychosocial laboratory stressor influences the cortisol stress response, with higher total but similar net cortisol responses in the morning compared to the afternoon (Kudielka et al., 2004) and lower cortisol responses in the evening (Yamanaka et al., 2019).

Numerous studies indicate strong links between circadian rhythms and the immune system. For example, peripheral clocks have been shown to be present in different hematopoietic cell lineages including macrophages and lymphocytes. Also, trafficking of the different types of immune cells in and out of the blood stream and the sensitivity to detect and respond to pathogens and their by-products, such as bacterial endotoxin, are under circadian control (Scheiermann et al., 2013). Many of the circadian effects on the immune system are mediated through direct innervation of sympathetic nerve fibers into lymphoid organs and by circulating stress hormones like catecholamines and glucocorticoids acting on α- and β-adrenergic and glucocorticoid receptors (GRs) (Dhabhar et al., 2012; Elenkov et al., 2000). The connection between the neuroendocrine stress system and the immune system is further supported by evidence that acute psychosocial stressors lead to an increase in circulating pro-inflammatory mediators (Prather et al., 2009; Steptoe et al., 2007) and an up-regulation of the transcription factor NF-κB, the master regulator of inflammation, in circulating immune cells (Bierhaus et al., 2003). Prior research has established the role of inflammatory mediators in the development of metabolic disturbances (Ndumele et al., 2006; Pradhan et al., 2001) and a recent study observed higher CRP concentrations, an acute-phase protein secreted by the liver in response to a variety of pro-inflammatory cytokines (Gabay, 2006), together with increased triglycerides levels and higher total body fat mass in evening vs morning- and intermediate types (Yu et al., 2015).

Both HPA-axis function and activation of pro-inflammatory pathways have been implicated in the development of adverse metabolic health outcomes. However, studies investigating the role of immune function in the context of chronobiology and (metabolic) health outcomes are scarce and until now, associations between chronotype and HPA-axis function have been mostly studied in the context of the circadian cortisol profile. Differences in stress-induced neuroendocrine reactivity patterns could also underlie the association between chronotype and metabolic function and may in parallel with prolonged activation of pro-inflammatory pathways contribute to the development of metabolic imbalances.

Thus, the first goal of the present study was to investigate the relationship between chronotype and two key interrelated biological systems that could potentially transmit the effect of chronotype on metabolic health outcomes: pro-inflammatory pathways, reflected by C-reactive protein concentrations, and the neuroendocrine stress response system, measured by heart rate and cortisol increase in response to acute psychosocial stress. In addition, we wanted to test the mediating and/or moderating roles of these systems in the association between chronotype and BMI, while adjusting for the possible confounding influence of sex (Kirschbaum et al., 1999) and sleep duration (Mota et al., 2016; Mullington et al., 2010; Spiegel et al., 2009; Van Lenten and Doane, 2016).

2. Materials and methods

2.1. Participants

Participants were recruited through announcements at universities in Berlin, Germany. To restrict the effect of age in our analyses we included subjects between 19–31 years old. In order to limit the effect of hormonal variation due to menstrual cycle on the neuroendocrine stress response only female subjects that were using oral contraceptives were included in the study. In addition, study visits were scheduled such that at the day of the TSST, none of the women were menstruating or during the 7 day break of their oral contraceptive intake. Additional exclusion criteria were CNS relevant diseases and neurological diseases, severe somatic diseases, diabetes type 1 and 2, steroid diseases, hypertonia, current infections, pregnancy, smoking and intake of medication (except for oral contraceptives in women). After screening 43 potential participants, the final study population comprised 28 young adults (14 women and 14 men, mean age 23.8 ± 3.3 (SD) years). All subjects were healthy as ascertained by self-report and confirmed by a brief clinical examination. BMI was assessed and calculated as weight in kilograms divided by height in meters squared (see Table 1 for participant characteristics and sample description). The study was approved by the medical ethics committee of Charité − Universitätsmedizin

Table 1
Participant characteristics, N = 28.

	N	Mean ± SD	Range
Gender			
Women	14 (50%)		
Men	14 (50%		
Age (years)		23.8 ± 3.3	19 – 31
BMI (kg/m^2)		20.9 ± 1.7	17.6 – 24.2
CRP (mg/L)		0.29 ± 0.41	0.019 – 1.97
Bed time (hh:mm)		11:40 pm ± 45 min	10:30 pm – 02:00 am
Wake time (hh:mm)		08:13 am ± 71 min	06:00 am – 12:00 pm
Sleep duration (h)		7.75 ± 0.99	5.5 – 11
PSQI		4.3 ± 2.29	1 – 10
MEQ score		50.6 ± 10.3	29 – 70
Chronotype			
Morning	5 (18%), 3 males (60%)		
Intermediate	19 (68%), 8 males (42.1%)		
Evening	4 (14%), 3 males (75%)		

Note. BMI, Body mass index; MEQ, Morningness-Eveningness Questionnaire; PSQI, Pittsburgh Sleep Quality Index.

Berlin and was conducted in accordance with the Declaration of Helsinki. All subjects provided written informed consent before participation.

2.2. Study protocol

Participants were asked to come to the laboratory at 2 p.m. and to refrain from eating at least 2.5 h before that time. Participants rested for 45 min and were then exposed to the Trier Social Stress Test (TSST). This laboratory-based protocol consists of a free speech task and a mental arithmetic task of 15 min duration performed in front of an audience and a camera (Kirschbaum et al., 1993). This protocol is among the most extensively validated tasks for the induction and assessment of acute psychophysiological stress responses, and has been found to induce significant endocrine (cortisol, ACTH), and sympathetic nervous system (as indexed by heart rate and blood pressure) responses in the vast majority of subjects (Dickerson and Kemeny, 2004). Saliva samples were obtained before (–10 min) and after (+30 min, +90 min) the TSST, whereas heart rate was measured continuously from –30 min before the start of the TSST to +45 min after the start of the TSST using an Actiheart device (Actiheart, CamNtech). Saliva was collected by placing cotton swabs (Salivettes, Sarstedt, Nümbrecht, Germany) in the participant's mouth for 2 min, and salivettes were immediately frozen at –80 °C. Blood samples to analyze CRP concentrations were collected before the TSST (–10 min) in EDTA containing vacutainers (BD Vacutainer) and immediately centrifuged at 4 °C for 10 min at 1500 x g. Plasma was then separated and stored at –80 °C.

2.3. Chronotype and sleep

Chronotype was assessed using the 19-item *Morningness-Eveningness Questionnaire* (MEQ) by Horne and Östberg (Horne and Ostberg, 1976). Analyses were performed with the continuous score of the MEQ. Higher values reflect morning tendency. Sleep quality was measured with the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989). The average effective sleep duration during the last month with question 4 from the PSQI: "How many hours did you usually sleep per day during the last month?" (Yu et al., 2015).

2.4. Salivary cortisol assay

Salivary cortisol concentrations were analyzed using a commercial ELISA kit (Salimetrics), with a sensitivity of 0.007 µg/dL. Intra-assay and inter-assay coefficients of variability were 6% and 7%, respectively.

2.5. Plasma CRP assay

Plasma CRP concentrations were analyzed using a commercially available high sensitivity Instant ELISA kit (eBioscience), according to the manufacturer's instructions. The limit of detection was 3 pg/mL. The intra- and inter-assay coefficients of variability for plasma CRP measurements were 6% and 8% respectively.

2.6. Statistical analyses

All variables were inspected for outliers, and if necessary log transformed to normalize the distribution (which was necessary only for CRP concentrations). Repeated-measures ANOVAs were computed to assess the effect of time on change in cortisol. Cortisol stress reactivity was determined by calculating the percent change in cortisol from before to 30 min after the TSST. For statistical analyses, heart rate was averaged across the following time windows: 5 min immediately before the onset of the TSST, 15 min during the TSST, and over a 5 min period from +40 min to +45 min after the onset of the TSST. T-tests were performed to test for differences between males and females. Pearson's correlations were used to test associations between the total MEQ score and participant characteristics, CRP and the cortisol and heart rate response. The PROCESS macro (Hayes, 2013) model 4 was used to assess the potential mediating roles of CRP concentrations and the cortisol response on the relationship between the MEQ score and BMI. We conducted ordinary least squares (OLS) path analyses using 10.000 bootstrapping samples and a bias-corrected 95% confidence interval (CI). To test for moderated mediation PROCESS model 14 was conducted. Variables were mean centered in order to easier interpret the interaction effect. As recommended by Preacher, Rucker, and Hayes (2007) (Preacher et al., 2007), high, medium and low levels of the moderator were operationalized at one standard deviation above and below the mean. The potentially confounding factors sex and sleep duration were included in all analyses. We also tested the potential influence of sleep quality in addition to sleep duration on the association between chronotype and BMI, CRP and the cortisol and heart rate response. Because sleep duration is part of the global PSQI sleep quality score, we ran separate models including sleep quality instead of sleep duration as a covariate. Adjusting for global PSQI score instead of sleep duration did not change the magnitude and significance of the reported associations. Data analysis was performed using the SPSS statistical software (SPSS 23.0, Inc., Chicago, IL, USA). The significance level was set at $p < .05$ for all applied analyses.

3. Results

3.1. Associations of MEQ score with participant characteristics, CRP and the stress response

Participant characteristics are presented in Table 1. There were no significant differences in MEQ scores between males and females ($t = –1.06$, $p > .05$). Additionally, evening tendency was associated with later bedtime ($r = –.39$, $p < .05$), but not with wake time ($r = –.17$, $p > .05$). The total MEQ score was significantly and negatively associated with BMI (Fig. 1a, $r = -.40$, $p < .05$), indicating that individuals with an evening tendency had a higher BMI. Lower total MEQ scores (i.e. evening tendency) were related to higher CRP concentrations ($r = -.42$, $p < .05$, Fig. 1b). In addition, BMI was positively associated with CRP concentrations ($r = .56$, $p < .01$).

As expected, exposure to the TSST led to significant changes in heart rate (main effect time: $F_{1.6,\ 36.6} = 80.7$, $p < .001$) and cortisol concentrations (–10, +30, +90 min, main effect time: $F_{1.5,\ 40.3} = 35.94$, $p < .001$, Fig. 2). Lower total MEQ scores (i.e. evening tendency) were associated with a higher TSST-induced cortisol response (%) (Fig. 1c, $r = -.53$, $p < .01$). Since higher basal levels of cortisol have been shown to be related to lower stress-related net increases in cortisol (Kudielka et al., 2004) we also controlled for baseline (–10 min) cortisol levels, but still observed a significant association between MEQ score and the cortisol stress response ($r = -.44$, $p < .05$). In addition, the cortisol response was positively associated to BMI ($r = .51$, $p < .01$) and CRP concentration ($r = .39$, $p < .05$). No significant relationship between MEQ score and the heart rate response ($r = –.039$, $p > .05$) was observed. Yet, evening tendency was associated with higher resting heart rate after the TSST ($r = –.44$, $p < .05$). Higher resting heart rate was also related to elevated CRP concentration ($r = .49$, $p < .05$) and a higher cortisol response ($r = .46$, $p < .05$), but was not significantly associated with BMI ($r = .23$, $p > .05$).

3.2. Mediation analyses

To gain a better understanding of the mechanisms underlying the relationship between chronotype and metabolic health outcomes, and as total MEQ score, BMI, CRP and the cortisol response were all correlated among each other, we first tested the possibility that the relationship between MEQ and BMI could be mediated by either CRP concentration or the cortisol response. Mediation analysis revealed that

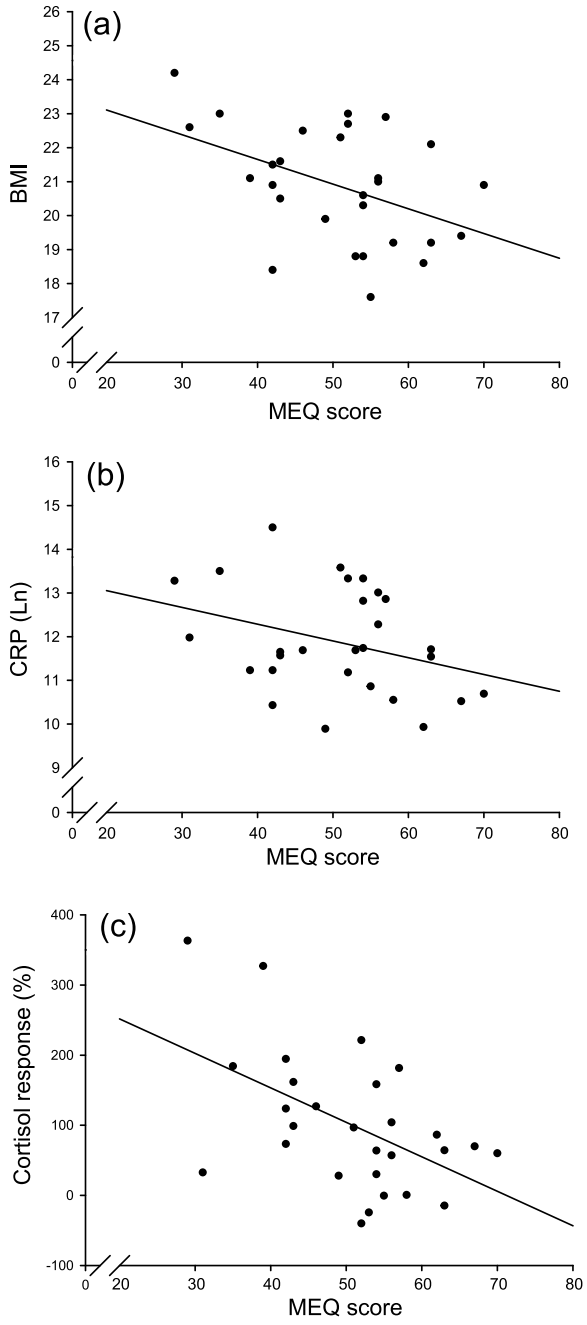


Fig. 1. Association of the total MEQ score with (a) BMI. (b) plasma CRP concentration pg/ml (Ln) and (c) the salivary cortisol response (%) after TSST induction. Lower MEQ scores suggest evening tendency. BMI, Body mass index; CRP, C-reactive protein, MEQ, Morningness-Eveningness Questionnaire.

the relationship between chronotype and BMI was mediated by CRP (b = –0.03, CI 95%: –0.08 to –0.007, p < .05), but not by the cortisol stress response (b = −0.03, CI 95%: –0.1 to 0.002, p > .05). In the first mediation model (including CRP as the mediator), adjusted for sex and sleep duration, evening tendency was related to higher CRP concentrations (b = −0.05, CI 95%: –0.09 to –0.005, p < .05), and CRP concentrations were positively associated with BMI (b = 0.63, CI 95%: 0.12 to 1.14, p < .05). Moreover, a lower score on the MEQ (i.e. evening tendency) was indirectly (b = –0.03, CI 95%: –0.08 to –0.007, p < .05), but not directly (b = –0.03, CI 95%: –0.09 to 0.03, p > .05), related to higher BMI. Overall, the model accounted for 48% of the variance in BMI (R^2 = .48, p < .01). This finding indicates that the higher BMI observed in individuals with a tendency towards evening

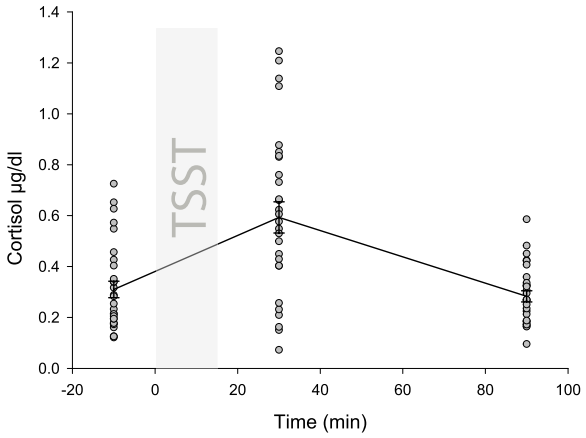


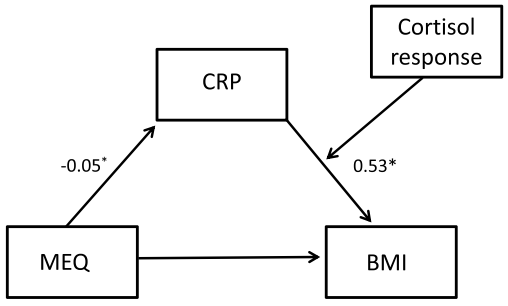
Fig. 2. Individual and mean (± SE) cortisol concentrations (µg/dl) before (–10 min) and after (+30 min, +90 min) the TSST (Trier Social Stress Test).

type may be explained at least in part by higher CRP levels.

Because we established significant associations between the cortisol stress response and the other measures that compose the above described mediation model, we further explored the possibility that the cortisol stress response moderates this mediated relationship. We observed that the effect of CRP on BMI was indeed contingent on the cortisol stress response, as evidenced by the statistically significant interaction between CRP and the cortisol response in the model of MEQ predicting BMI (b = 0.005, CI 95%: 0.0002 to 0.01, p < .05), such that the mediated relationship is stronger when the cortisol response is higher (see also Fig. 3). Table 2 shows that the conditional indirect effect for MEQ on BMI was significant when the cortisol response was of average or high magnitude (mean or above one standard deviation (SD) of the mean), but not when the cortisol response was of lower magnitude (mean – 1 SD).

4. Discussion

In the present study we aimed to investigate the mechanisms underlying the relationship between chronotype and metabolic health outcomes. The major findings of our study are the following: First, our findings replicate previous results indicating that evening tendency is



Direct effect, b = -0.006, 95% CI [-0.06, 0.05]

Indirect Effect,
Low cortisol response, b = -0.002, 95% CI [-0.05, 0.04]
Average cortisol response, b = -0.03, 95% CI [-0.07, -0.005]
High cortisol response, b = -0.05, 95% CI [-0.12, -0.01]

Fig. 3. Results from the moderated mediation model including, MEQ global score, CRP, BMI and the cortisol response adjusted for sex and sleep duration. Unstandarized regression coefficients are displayed on the arrows. * indicates p-value < .05. BMI, Body mass index; CRP, C-reactive protein; MEQ, Morningness-Eveningness Questionnaire.

Table 2
Conditional indirect effects of MEQ score on BMI at different values of the cortisol response.

Cortisol response	Effect	SE	95% CI
−97.7 (mean -1 SD)	−0.002	0.02	−0.05 to 0.04
0 (mean)	−0.03	0.02	−0.07 to –0.005
97.7 (mean +1 SD)	−0.05	0.03	−0.12 to –0.01

Note. Variables were mean centered in order to easier interpret the interaction effect. The conditional indirect effect for MEQ on BMI was significant when the cortisol response was of average or high magnitude (mean or mean + 1 SD), but not when the cortisol response was of lower magnitude (mean – 1 SD). BMI, Body mass index; CI, Confidence interval, MEQ, Morningness-Eveningness Questionnaire.

associated with higher BMI (Arora and Taheri, 2014; Lucassen et al., 2013); second, we observed elevated plasma CRP concentrations and an increased cortisol stress response after a standardized laboratory stressor in late individuals; third, our results suggest that the relationship between chronotype and BMI is mediated by elevated CRP concentration and that this mediated relationship is stronger in individuals with an increased cortisol response. Altogether, these results imply that elevated circulating CRP concentration in combination with a higher cortisol stress response could underlie the development of metabolic dysfunction in individuals with a tendency towards the evening chronotype.

In line with previous results our findings indicate that evening tendency is associated with higher BMI (Arora and Taheri, 2014; Lucassen et al., 2013). Individual differences in the total MEQ score accounted for 16% of the observed variation in BMI. An earlier study by Lucassen et al. (Lucassen et al., 2013) found that about 6% of the variation in BMI could be explained by the total MEQ score, yet, this study was conducted in obese individuals. In accordance with our findings, prior research has shown that plasma CRP concentration is increased in the late or evening chronotype (Yu et al., 2015), an effect that could possibly be explained by alterations in autonomic nervous system activity (Roeser et al., 2012). As previously reported, in our study evening tendency (Lucassen et al., 2013; Roeser et al., 2012) and CRP concentrations (Whelton et al., 2014) were both associated with higher resting heart rate. The autonomic nervous system directly regulates cellular inflammatory signaling, and increased sympathetic nervous system activity has been associated with elevated inflammation (de Punder and Pruimboom, 2015). Also, evening type is associated with less favorable dietary habits. For example, evening types consume more calories (Mota et al., 2016), fast food and sweets and eat less fish, fruits and vegetables compared to the early and intermediate chronotypes (Arora and Taheri, 2014; Fleig and Randler, 2009; Kanerva et al., 2012). Food intake can produce post-prandial immune activation when a meal is high in calories or has a high fat content (de Punder and Pruimboom, 2015). Also, evening tendency is related to later meal times (Lucassen et al., 2013). A recent study found significant associations between evening caloric intake (assessed between 5 pm and midnight) and circulating concentrations of CRP (Marinac et al., 2015).

In addition, this study provides the first evidence in humans suggesting that the effect of chronotype on BMI is mediated by elevated plasma CRP concentration. Inflammation is closely related to adiposity; adipose cells are known to secrete pro-inflammatory mediators when the adipose tissue becomes hypertrophic and subsequently hyperplastic as a consequence of metabolic dysregulation (Rehman and Akash, 2016). However, the relationship between inflammation and adiposity is bidirectional. For example, several studies observed strong relationships between inflammatory mediators, among which CRP, and the development of insulin resistance, an important contributor in the development of metabolic disturbances (Ndumele et al., 2006; Pradhan et al., 2001). By altering insulin receptor substrate proteins, pro-inflammatory pathways impair insulin signaling in adipose tissues at the level of the insulin receptor (Nieto-Vazquez et al., 2008).

Our finding that evening tendency is associated with a higher cortisol response to a standardized psychosocial laboratory stressor (TSST) is in contrast to a recent study reporting increased TSST-induced cortisol responses in morning compared to evening types (Marvel-Coen et al., 2018). This discrepancy could possibly be explained by the applied version of the MEQ; we used the original 19-item version of the MEQ, while Marvel-Coen et al. (Marvel-Coen et al., 2018) applied the reduced 5-item version. Another explanation could be that, in contrast to the other study, we adjusted for the effect of possible confounders (sex and sleep duration) in all the studied relationships. Based on our observations and previously published findings showing that resting heart rate is elevated in the late chronotype (Lucassen et al., 2013; Roeser et al., 2012), the increased cortisol response induced by the TSST in late individuals might possibly be explained by alterations in autonomic nervous system function. Additionally, evening types have reported higher levels of perceived psychosocial stress compared to the morning- and intermediate chronotypes (Kantermann et al., 2012).

We observed a positive relationship between the cortisol stress response and BMI. This finding is in agreement with previous studies. For example, a systematic review indicated that obese individuals reliably demonstrate higher cortisol reactivity in response to an acute stressor (Incollingo Rodriguez et al., 2015). Glucocorticoids regulate lipid metabolism in adipose tissue and the liver, stimulate hepatic gluconeogenesis and induce short-term insulin resistance in the periphery (Hitze et al., 2010) and, therefore, prolonged activation of the HPA-axis could promote abdominal visceral fat accumulation as well as increase body weight via increased cortisol secretion (Geiker et al., 2018). In addition, prior research showed that individuals who were offered a rich buffet after an acute social laboratory stressor (TSST) stated less burdening during social stress exposition than subjects who were offered a meager salad (Hitze et al., 2010), suggesting that individuals that exhibit higher stress responses could be more inclined to eat “comfort food” after being exposed to daily stressors. Also, individuals showing higher acute stress-induced increases in cortisol exhibited a stronger repression of plasma insulin levels and showed steeper increases in glucose concentrations following rich buffet ingestion after the stressor (Hitze et al., 2010), further indicating that HPA-axis reactivity patterns influence metabolic function.

Additionally, we observed a moderating effect of the cortisol stress response on the relationship between CRP and BMI. Enhanced stress responsiveness may be induced by changes in glucocorticoid receptor (GR) mediated feedback control at the level of the HPA-axis and the immune system. Normally, the release of glucocorticoids puts a limit on the maximum activity of the immune system, yet, impaired functioning of the GR could make the immune system insensitive to its inhibitory and modulatory actions (Barnes, 2017). Inflammatory mediators, in their turn, can further stimulate sympathetic nervous system and HPA-axis responses (Dunn, 2000; Goebel et al., 2002; Zimomra et al., 2011), creating a vicious circle, resulting in less functional GRs and a pro-inflammatory state (Barnes, 2017). Chronic activation of pro-inflammatory pathways induces insulin resistance and promotes the development of metabolic disturbances (Ndumele et al., 2006; Pradhan et al., 2001). Also, animal studies indicated that deficiencies in glucocorticoid receptor functioning can provoke disruptions in the circadian rhythm (Chung et al., 2011).

Later chronotype is associated with a greater degree of misalignment of biological and social time, which could result in the accumulation of a substantial sleep debt in the evening chronotype (Wittmann et al., 2006). Previous studies have shown associations between chronotype and sleep duration (Mota et al., 2016) and between sleep duration and metabolic function (Spiegel et al., 2009), inflammation (Mullington et al., 2010) and the functioning of the HPA-axis (Van Lenten and Doane, 2016). Therefore, here we adjusted all analyses for self-reported sleep duration, which suggests that our findings are not explained by self-reported hours of sleep in the last month.

Limitations of our study include the cross sectional design, the relatively modest sample size and the restricted age and BMI range in our study sample. In addition we only assessed BMI and did not include measures of waist circumference or adiposity. Replication of our results in a sample that includes overweight (BMI 25–29.9) or obese (BMI ≥ 30) individuals is warranted. Another limitation of the study is that we measured chronotype by questionnaire and not included an objective measure of circadian phase, like the dim light melatonin onset. We also did not assess participants' eating habits that might at least partly explain our results. Finally, all participants were exposed to the TSST in the afternoon. It might well be that individuals of a certain chronotype show different physiological stress responses when tested at different times of the day. Therefore, future studies should attempt to identify the effect of time of day of TSST exposure on the relationships between the cortisol stress response, BMI and inflammation in the different chronotypes.

Taken together, the current findings advance our understanding of the underlying role of the immune system and the HPA-axis in the relationship between chronobiology and metabolic health outcomes.

Author contributions

K.d.P. coordinated the study, participated in the design of the study, carried out the laboratory assays, participated in the data analysis and drafted the manuscript. C.H. participated in the data analysis and provided editorial assistance. S.E. conceived of and designed the study, participated in the analysis and interpretation of study findings, drafted portions of the manuscript and provided final editorial oversight.

Disclosure statement

The authors have nothing to disclose.

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Declaration of Competing Interest

The authors declare no conflict of interest.

References

Adan, A., Guardia, J., 1993. Circadian variations of self-reported activation: a multidimensional approach. Chronobiologia 20, 233–244.
Akerstedt, T., Froberg, J.E., 1976. Interindividual differences in circadian patterns of catecholamine excretion, body temperature, performance, and subjective arousal. Biol. Psychol. 4, 277–292.
Arora, T., Taheri, S., 2014. Associations among late chronotype, body mass index and dietary behaviors in young adolescents. Int. J. Obes. 39, 39.
Bailey, S.L., Heitkemper, M.M., 2001. Circadian rhythmicity of cortisol and body temperature: morningness-eveningness effects. Chronobiol. Int. 18, 249–261.
Barnes, P.J., 2017. Glucocorticosteroids. Handb. Exp. Pharmacol. 237, 93–115.
Bierhaus, A., Wolf, J., Andrassy, M., Rohleder, N., Humpert, P.M., Petrov, D., Ferstl, R., von Eynatten, M., Wendt, T., Rudofsky, G., Joswig, M., Morcos, M., Schwaninger, M., McEwen, B., Kirschbaum, C., Nawroth, P.P., 2003. A mechanism converting psychosocial stress into mononuclear cell activation. Proc. Natl. Acad. Sci. U.S.A. 100, 1920–1925.
Bonato, M., La Torre, A., Saresella, M., Marventano, I., Merati, G., Vitale, J.A., 2017. Salivary cortisol concentration after high-intensity interval exercise: time of day and chronotype effect. Chronobiol. Int. 34, 698–707.
Buysse, D.J., Reynolds 3rd, C.F., Monk, T.H., Berman, S.R., Kupfer, D.J., 1989. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 28, 193–213.
Chung, S., Son, G.H., Kim, K., 2011. Circadian rhythm of adrenal glucocorticoid: its regulation and clinical implications. Biochim. Biophys. Acta 1812, 581–591.
de Punder, K., Pruimboom, L., 2015. Stress induces endotoxemia and low-grade inflammation by increasing barrier permeability. Front. Immunol. 6, 223.
Dhabhar, F.S., Malarkey, W.B., Neri, E., McEwen, B.S., 2012. Stress-induced redistribution of immune cells–from barracks to boulevards to battlefields: a tale of three hormones–Curt Richter Award winner. Psychoneuroendocrinology 37, 1345–1368.
Dickerson, S.S., Kemeny, M.E., 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Psychol. Bull. 130, 355–391.
Dockray, S., Steptoe, A., 2011. Chronotype and diurnal cortisol profile in working women: differences between work and leisure days. Psychoneuroendocrinology 36, 649–655.
Dunn, A.J., 2000. Cytokine activation of the HPA axis. Ann. N. Y. Acad. Sci. 917, 608–617.
Elenkov, I.J., Wilder, R.L., Chrousos, G.P., Vizi, E.S., 2000. The sympathetic nerve–an integrative interface between two supersystems: the brain and the immune system. Pharmacol. Rev. 52, 595–638.
Fleig, D., Randler, C., 2009. Association between chronotype and diet in adolescents based on food logs. Eat. Behav. 10, 115–118.
Gabay, C., 2006. Interleukin-6 and chronic inflammation. Arthritis Res. Ther. 8 (Suppl 2), S3.
Geiker, N.R.W., Astrup, A., Hjorth, M.F., Sjodin, A., Pijls, L., Markus, C.R., 2018. Does stress influence sleep patterns, food intake, weight gain, abdominal obesity and weight loss interventions and vice versa? Obes. Rev. 19, 81–97.
Goebel, M.U., Baase, J., Pithan, V., Exton, M., Saller, B., Schedlowski, M., Limmroth, V., 2002. Acute interferon beta-1b administration alters hypothalamic-pituitary-adrenal axis activity, plasma cytokines and leukocyte distribution in healthy subjects. Psychoneuroendocrinology 27, 881–892.
Hayes, A.F., 2013. Introduction to Mediation, Moderation, and Conditional Process Analysis: A Regression-Based Approach. The Guilford Press The Guilford Press New York, New York, NY.
Hitze, B., Hubold, C., van Dyken, R., Schlichting, K., Lehnert, H., Entringer, S., Peters, A., 2010. How the selfish brain organizes its supply and demand. Front. Neuroenergetics 2, 7.
Horne, J.A., Ostberg, O., 1976. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int. J. Chronobiol. 4, 97–110.
Huang, W., Ramsey, K.M., Marcheva, B., Bass, J., 2011. Circadian rhythms, sleep, and metabolism. J. Clin. Invest. 121, 2133–2141.
Incollingo Rodriguez, A.C., Epel, E.S., White, M.L., Standen, E.C., Seckl, J.R., Tomiyama, A.J., 2015. Hypothalamic-pituitary-adrenal axis dysregulation and cortisol activity in obesity: a systematic review. Psychoneuroendocrinology 62, 301–318.
Ishihara, K., Miyasita, A., Inugami, M., Fukuda, K., Miyata, Y., 1987. Differences in sleep-wake habits and EEG sleep variables between active morning and evening subjects. Sleep 10, 330–342.
Joseph, J.J., Golden, S.H., 2017. Cortisol dysregulation: the bidirectional link between stress, depression, and type 2 diabetes mellitus. Ann. N. Y. Acad. Sci. 1391, 20–34.
Kanerva, N., Kronholm, E., Partonen, T., Ovaskainen, M.L., Kaartinen, N.E., Konttinen, H., Broms, U., Mannisto, S., 2012. Tendency toward eveningness is associated with unhealthy dietary habits. Chronobiol. Int. 29, 920–927.
Kantermann, T., Theadom, A., Roenneberg, T., Cropley, M., 2012. Fibromyalgia syndrome and chronotype: late chronotypes are more affected. J. Biol. Rhythms 27, 176–179.
Kerkhof, G.A., Van Dongen, H.P., 1996. Morning-type and evening-type individuals differ in the phase position of their endogenous circadian oscillator. Neurosci. Lett. 218, 153–156.
Kirschbaum, C., Kudielka, B.M., Gaab, J., Schommer, N.C., Hellhammer, D.H., 1999. Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. Psychosom. Med. 61, 154–162.
Kirschbaum, C., Pirke, K.M., Hellhammer, D.H., 1993. The' Trier Social Stress Test'–a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28, 76–81.
Kudielka, B.M., Bellingrath, S., Hellhammer, D.H., 2007. Further support for higher salivary cortisol levels in "morning" compared to "evening" persons. J. Psychosom. Res. 62, 595–596.
Kudielka, B.M., Federenko, I.S., Hellhammer, D.H., Wust, S., 2006. Morningness and eveningness: the free cortisol rise after awakening in "early birds" and "night owls". Biol. Psychol. 72, 141–146.
Kudielka, B.M., Schommer, N.C., Hellhammer, D.H., Kirschbaum, C., 2004. Acute HPA axis responses, heart rate, and mood changes to psychosocial stress (TSST) in humans at different times of day. Psychoneuroendocrinology 29, 983–992.
Lucassen, E.A., Zhao, X., Rother, K.I., Mattingly, M.S., Courville, A.B., de Jonge, L., Csako, G., Cizza, G., Extension, Sleep, Study, G., 2013. Evening chronotype is associated with changes in eating behavior, more sleep apnea, and increased stress hormones in short sleeping obese individuals. PLoS One 8, e56519.
Marinac, C.R., Sears, D.D., Natarajan, L., Gallo, L.C., Breen, C.I., Patterson, R.E., 2015. Frequency and circadian timing of eating may influence biomarkers of inflammation and insulin resistance associated with breast Cancer risk. PLoS One 10, e0136240.
Marvel-Coen, J., Nickels, N., Maestripieri, D., 2018. The relationship between morningness–eveningness, psychosocial variables, and cortisol reactivity to stress from a life history perspective. Evol. Behav. Sci. 12, 71–86.
Merikanto, I., Englund, A., Kronholm, E., Laatikainen, T., Peltonen, M., Vartiainen, E., Partonen, T., 2014. Evening chronotypes have the increased odds for bronchial asthma and nocturnal asthma. Chronobiol. Int. 31, 95–101.
Merikanto, I., Lahti, T., Kronholm, E., Peltonen, M., Laatikainen, T., Vartiainen, E., Salomaa, V., Partonen, T., 2013a. Evening types are prone to depression. Chronobiol. Int. 30, 719–725.
Merikanto, I., Lahti, T., Puolijoki, H., Vanhala, M., Peltonen, M., Laatikainen, T., Vartiainen, E., Salomaa, V., Kronholm, E., Partonen, T., 2013b. Associations of chronotype and sleep with cardiovascular diseases and type 2 diabetes. Chronobiol. Int. 30, 470–477.

Monk, T.H., Buysse, D.J., Potts, J.M., DeGrazia, J.M., Kupfer, D.J., 2004. Morningness-eveningness and lifestyle regularity. Chronobiol. Int. 21, 435–443.
Mota, M.C., Waterhouse, J., De-Souza, D.A., Rossato, L.T., Silva, C.M., Araujo, M.B., Tufik, S., de Mello, M.T., Crispim, C.A., 2016. Association between chronotype, food intake and physical activity in medical residents. Chronobiol. Int. 33, 730–739.
Mullington, J.M., Simpson, N.S., Meier-Ewert, H.K., Haack, M., 2010. Sleep loss and inflammation. Best Pract. Res. Clin. Endocrinol. Metab. 24, 775–784.
Ndumele, C.E., Pradhan, A.D., Ridker, P.M., 2006. Interrelationships between inflammation, C-reactive protein, and insulin resistance. J. Cardiometab. Syndr. 1, 190–196.
Nieto-Vazquez, I., Fernandez-Veledo, S., Kramer, D.K., Vila-Bedmar, R., Garcia-Guerra, L., Lorenzo, M., 2008. Insulin resistance associated to obesity: the link TNF-alpha. Arch. Physiol. Biochem. 114, 183–194.
Olds, T.S., Maher, C.A., Matricciani, L., 2011. Sleep duration or bedtime? Exploring the relationship between sleep habits and weight status and activity patterns. Sleep 34, 1299–1307.
Parsons, M.J., Moffitt, T.E., Gregory, A.M., Goldman-Mellor, S., Nolan, P.M., Poulton, R., Caspi, A., 2015. Social jetlag, obesity and metabolic disorder: investigation in a cohort study. Int. J. Obes. (Lond) 39, 842–848.
Pradhan, A.D., Manson, J.E., Rifai, N., Buring, J.E., Ridker, P.M., 2001. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 286, 327–334.
Prather, A.A., Carroll, J.E., Fury, J.M., McDade, K.K., Ross, D., Marsland, A.L., 2009. Gender differences in stimulated cytokine production following acute psychological stress. Brain Behav. Immun. 23, 622–628.
Preacher, K.J., Rucker, D.D., Hayes, A.F., 2007. Addressing moderated mediation hypotheses: theory, methods, and prescriptions. Multivariate Behav. Res. 42, 185–227.
Randler, C., Schaal, S., 2010. Morningness-eveningness, habitual sleep-wake variables and cortisol level. Biol. Psychol. 85, 14–18.
Rehman, K., Akash, M.S., 2016. Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked? J. Biomed. Sci. 23, 87.
Roenneberg, T., Allebrandt, K.V., Merrow, M., Vetter, C., 2012. Social jetlag and obesity. Curr. Biol. 22, 939–943.
Roeser, K., Obergfell, F., Meule, A., Vogele, C., Schlarb, A.A., Kubler, A., 2012. Of larks and hearts–morningness/eveningness, heart rate variability and cardiovascular stress response at different times of day. Physiol. Behav. 106, 151–157.
Scheiermann, C., Kunisaki, Y., Frenette, P.S., 2013. Circadian control of the immune system. Nat. Rev. Immunol. 13, 190–198.
Spiegel, K., Tasali, E., Leproult, R., Van Cauter, E., 2009. Effects of poor and short sleep on glucose metabolism and obesity risk. Nat. Rev. Endocrinol. 5, 253–261.
Steptoe, A., Hamer, M., Chida, Y., 2007. The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis. Brain Behav. Immun. 21, 901–912.
Uusitalo, A., Ahonen, J.P., Gorski, P., Tuomisto, M., Turjanmaa, V., 1988. Does the biorhythm of morningness or eveningness predict the arterial blood pressure level? Ann. Clin. Res. 20 (Suppl 48), 51–53.
Van Lenten, S.A., Doane, L.D., 2016. Examining multiple sleep behaviors and diurnal salivary cortisol and alpha-amylase: within- and between-person associations. Psychoneuroendocrinology 68, 100–110.
Whelton, S.P., Narla, V., Blaha, M.J., Nasir, K., Blumenthal, R.S., Jenny, N.S., Al-Mallah, M.H., Michos, E.D., 2014. Association between resting heart rate and inflammatory biomarkers (high-sensitivity C-reactive protein, interleukin-6, and fibrinogen) (from the Multi-Ethnic Study of Atherosclerosis). Am. J. Cardiol. 113, 644–649.
Wittmann, M., Dinich, J., Merrow, M., Roenneberg, T., 2006. Social jetlag: misalignment of biological and social time. Chronobiol. Int. 23, 497–509.
Yamanaka, Y., Motoshima, H., Uchida, K., 2019. Hypothalamic-pituitary-adrenal axis differentially responses to morning and evening psychological stress in healthy subjects. Neuropsychopharmacol Rep 39, 41–47.
Yu, J.H., Yun, C.H., Ahn, J.H., Suh, S., Cho, H.J., Lee, S.K., Yoo, H.J., Seo, J.A., Kim, S.G., Choi, K.M., Baik, S.H., Choi, D.S., Shin, C., Kim, N.H., 2015. Evening chronotype is associated with metabolic disorders and body composition in middle-aged adults. J. Clin. Endocrinol. Metab. 100, 1494–1502.
Zimomra, Z.R., Porterfield, V.M., Camp, R.M., Johnson, J.D., 2011. Time-dependent mediators of HPA axis activation following live Escherichia coli. Am. J. Physiol. Regul. Integr. Comp. Physiol. 301, R1648–1657.