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Influence of sleep deprivation and circadian misalignment on cortisol, inflammatory markers, and cytokine balance

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ABSTRACT

Cortisol and inflammatory proteins are released into the blood in response to stressors and chronic elevations of blood cortisol and inflammatory proteins may contribute to ongoing disease processes and could be useful biomarkers of disease. How chronic circadian misalignment influences cortisol and inflammatory proteins, however, is largely unknown and this was the focus of the current study. Specifically, we examined the influence of weeks of chronic circadian misalignment on cortisol, stress ratings, and pro- and anti-inflammatory proteins in humans. We also compared the effects of acute total sleep deprivation and chronic circadian misalignment on cortisol levels. Healthy, drug free females and males ($N = 17$) aged 20–41 participated. After 3 weeks of maintaining consistent sleep–wake schedules at home, six laboratory baseline days and nights, a 40-h constant routine (CR, total sleep deprivation) to examine circadian rhythms for melatonin and cortisol, participants were scheduled to a 25-day laboratory entrainment protocol that resulted in sleep and circadian disruption for eight of the participants. A second constant routine was conducted to reassess melatonin and cortisol rhythms on days 34–35. Plasma cortisol levels were also measured during sampling windows every week and trapezoidal area under the curve (AUC) was used to estimate 24-h cortisol levels. Inflammatory proteins were assessed at baseline and near the end of the entrainment protocol. Acute total sleep deprivation significantly increased cortisol levels ($p < 0.0001$), whereas chronic circadian misalignment significantly reduced cortisol levels ($p < 0.05$). Participants who exhibited normal circadian phase relationships with the wakefulness–sleep schedule showed little change in cortisol levels. Stress ratings increased during acute sleep deprivation ($p < 0.0001$), whereas stress ratings remained low across weeks of study for both the misaligned and synchronized control group. Circadian misalignment significantly increased plasma tumor necrosis factor-alpha (TNF-α), interleukin 10 (IL-10) and C-reactive protein (CRP) ($p < 0.05$). Little change was observed for the TNF-α/IL-10 ratio during circadian misalignment, whereas the TNF-α/IL-10 ratio and CRP levels decreased in the synchronized control group across weeks of circadian entrainment. The current findings demonstrate that total sleep deprivation and chronic circadian misalignment modulate cortisol levels and that chronic circadian misalignment increases plasma concentrations of pro- and anti-inflammatory proteins.

1. Introduction

The internal circadian clock and sleep–wakefulness physiology modulate daily patterns in most behavioral and physiological systems 1 (Bass and Takahashi, 2010; Czeisler and Klerman, 1999; Davies et al., 2014; Wright et al., 2012). Insufficient sleep and circadian misalignment have negative impacts on endocrine, metabolic, cardiovascular, immune, bone, stress, cognition, and neurological health and function (Depner et al., 2014; Dimitrov et al., 2004; Everson et al., 2012; Everson and Szabo, 2011; Haack et al., 2004; Lekander et al., 2013; Markwald et al., 2013; Scheer

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et al., 2009; Spiegel et al., 1999; Thompson et al., 2014; Weil et al., 2013; Wright et al., 2006; Yu et al., 2013). Sleep deprivation is considered a physiological stressor and a metabolic challenge that is often associated with increased cortisol levels and stress ratings (Chapotot et al., 2001; Dinges et al., 1997; Leproult et al., 1997; Minkel et al., 2012; Parry et al., 2000; Spiegel et al., 1999; von Treuer et al., 1996; Weibel et al., 1995; Weitzman et al., 1983). Sleep loss is also reported to elevate blood concentrations of inflammatory proteins and may be reflective of impaired physiological function and disease processes (Irwin et al., 2010; Mullington et al., 2010). While much is known about the influence of insufficient sleep on stress, cortisol, inflammation and the risk of impaired heath and disease in humans, less is known about the influence of chronic circadian misalignment on cortisol and inflammatory proteins. Circadian misalignment results when sleep and wakefulness occur at inappropriate circadian times; i.e., when wakefulness occurs at a time the internal circadian clock is promoting sleep and/or when sleep occurs at a time when the internal clock is promoting wakefulness (Baron and Reid, 2014; Gronfier et al., 2007; Wright et al., 2006). Circadian misalignment can be acute such as during total sleep deprivation (Frey et al., 2004; McHill et al., 2014), intermittent as during shift work and jet lag (Sack et al., 2007a; Wright et al., 2013; Zee et al., 2010), or chronic as in circadian rhythm sleep–wake disorders (Sack et al., 2007a,b).

The daily pattern of the endocrine hormone cortisol is strongly driven by the master circadian clock, located in the suprachiasmatic nucleus (SCN) of the hypothalamus (Moore and Eichler, 1972). The circadian clock modulates the near-24-hour rhythm in cortisol via the hypothalamic–pituitary–adrenal (HPA) axis and via neural innervation through a polysynaptic pathway from the SCN to the autonomic area of the paraventricular nucleus of the hypothalamus and the spinal cord (Buijs et al., 1999) providing sympathetic innervation (Buijs et al., 2003). The circadian rhythm in cortisol shows high levels in the morning near habitual waketime in humans, declines across the biological day, shows low levels in the early evening and increases across the biological night (Czeisler and Klerman, 1999; Desir et al., 1980; Van Cauter et al., 1994). The cortisol rhythm can thus be used as a phase marker of the circadian clock (Desir et al., 1980; Van Cauter and Refetoff, 1985). Factors such as stress (Morgan et al., 2001; Stratakis and Chrousos, 1995), meals (Follenius et al., 1982; Ishizuka et al., 1983), exercise (Brandenberger and Follenius, 1975), and awakening from sleep (Gribbin et al., 2012) induce acute increases in cortisol levels and factors such as sleep (Gronfier et al., 1998, 1997, 1999; Weibel et al., 1995) and bright light exposure (Jung et al., 2010) can induce acute decreases of cortisol levels.

Daily patterns of immune factors and responses to immune challenge are modulated by sleep and circadian phase (Curtis et al., 2014; Fonken et al., 2013; Gibbs et al., 2012; Keller et al., 2009; Moller-Levet et al., 2013; Morrow and Opp, 2005; Narasimamurthy et al., 2012; Pollmacher et al., 1996; Rahman et al., 2014). Immune factors contribute to the natural sleep process (Imeri and Opp, 2009; Krueger et al., 2011; Marshall and Born, 2002) and sleep and circadian disruption are reported to alter inflammatory proteins (Axelsson et al., 2013; Chennaoui et al., 2011; Fondell et al., 2011; Frey et al., 2007; Haack et al., 2007; Meier-Ewert et al., 2004; Mullington et al., 2010; Redwine et al., 2000; Shearer et al., 2001). Most prior studies of how circadian disruption in humans influences inflammation however, are limited methodologically by infrequent sampling rates, typically sampling at only one or a few time points across the 24-h day (Copertaro et al., 2011; Khosro et al., 2011; Puttonen et al., 2011; Sookoian et al., 2007) and limited inflammatory protein assessment. One notable exception regarding sampling rate is a study in which C-reactive protein (CRP) was examined every 4 h over 24-h at baseline and on day 8 of sleep restriction during which days 2-3 and 5-6 the participants were also circadian misaligned by scheduling sleep during the daytime (Leproult et al., 2014). As sleep–wakefulness state and circadian phase modulate immune function, additional studies with frequent sampling of multiple inflammatory proteins and concurrent assessment of other biological factors that influence inflammation, such as endogenous cortisol (Yeager et al., 2011), are needed to improve our understanding of immune changes associated with circadian disruption. How cortisol and inflammatory proteins are influenced by chronic circadian misalignment is largely unknown. Therefore, the focus of the current analyses was to determine the influence of chronic circadian misalignment on cortisol and frequently sampled inflammatory proteins including the pro-inflammatory proteins tumor necrosis factor alpha (TNF-α) and CRP and the anti-inflammatory cytokine interleukin-10 (IL-10). The current analysis also compared the influence of chronic circadian misalignment to the influence of acute total sleep deprivation on cortisol levels. As noted, because stress increases cortisol levels (Morgan et al., 2001; Stratakis and Chrousos, 1995), the current study also examined changes in stress ratings across total sleep deprivation and chronic circadian misalignment.

2. Methods

Detailed methods and circadian melatonin phase, sleep, leptin, and performance findings from the studies presented here have been published (Nguyen and Wright, 2010; Wright et al., 2001, 2006). The current manuscript represents planned analyses for cortisol, inflammatory proteins and stress ratings.

2.1. Participant screening and pre-laboratory conditions

We studied healthy females and males (N = 17 [3 females]) aged 31.7 ± 6.1 (Mean ± SD). Participants gave written informed consent and the Partners Health Care (Boston, MA) and the University of Colorado Boulder Institutional Review Boards approved the procedures and/or analyses for the protocol. Data collection was conducted at the Brigham and Women's Hospital. All participants were determined to be healthy after passing a rigorous health screening, including medical history, physical exam, electrocardiogram, blood and urine chemistries, a toxicology screen for drug use, psychological tests and an interview with a clinical psychologist. None reported regular night work or rotating shift work within the past three years or crossing more than one time zone in the previous three months. Participants maintained a regular routine of 8-h scheduled sleep and 16-h scheduled wakefulness for a minimum of 3 weeks while living at home before the in-laboratory protocol, as verified by sleep logs, call-in times to a time stamped voice recorder and wrist actigraphy recordings for at least 1 week prior to laboratory admission (Philips Respironics, Mini Mitter, Bend OR).

2.2. In-laboratory conditions

Participants were tested individually in an environment free of time cues. Ambient light, room temperature, sleep–wakefulness opportunities, activity, and nutrition intake (breakfast, lunch, dinner and a snack; 150 mEq Na^{++}, 100 mEq K^{+} ± 20%, 1500 to 2500 cc fluids, isocaloric) were strictly controlled. Exercise and napping were proscribed. Participants were initially scheduled to a 16-h wakefulness 8-h sleep schedule for 6 days at their habitual wakefulness–sleep times (Fig. 1). Habitual bedtime was calculated by subtracting 4 h from the average midpoint of the participants' self-selected wakefulness–sleep schedule during the week prior to laboratory admission. Following the 6 baseline days, an initial

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