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Nocturnal levels of ghrelin and leptin and sleep in chronic insomnia

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Summary Experimental sleep deprivation in healthy humans affects levels of ghrelin and leptin, two primary hormones involved in energy balance that regulate appetite and body weight. No study to date has examined levels of these hormones in patients with chronic insomnia. In this study, men diagnosed with primary insomnia using DSM-IV criteria ($n = 14$) and age and body weight comparable healthy control men ($n = 24$) underwent polysomnography. Circulating levels of ghrelin and leptin were measured at 2300 h, 0200 h and 0600 h. As compared to controls, insomnia patients showed less total sleep time, stage 2 and REM sleep and decreased sleep efficiency and more stage 1 sleep than controls (p's $< .05$). Ghrelin levels across the night were significantly lower in insomnia patients ($p < .0001$). Leptin was not significantly different between the groups. In conclusion, decreased nocturnal ghrelin in insomnia is consistent with findings for nighttime levels in sleep deprivation studies in healthy sleepers. These findings suggest that insomnia patients have a dysregulation in energy balance that may play a role in explaining prospective weight gain in this population.

1. Introduction

Recent studies have linked hormones associated with energy balance such as ghrelin and leptin with sleep. In both experimental and naturalistic studies, restricted or inadequate sleep is associated with altered levels of ghrelin and leptin (Mullington et al., 2003; Spiegel et al., 2004; Taheri et al., 2004), spurring the theory that poor sleep may disrupt endocrine regulation of energy balance, promoting weight gain (Cummings et al., 2005). Chronic insomnia is associated with obesity (Foley et al., 1995; Taheri et al., 2004) and prospectively predicts weight gain (Janson et al., 2001; Hasler et al., 2004). No study to date has evaluated nocturnal levels of ghrelin and leptin in primary insomnia patients.

Ghrelin and leptin signal the brain regarding bodily state of energy balance promoting either satiety or hunger (Muccioli et al., 2002). Ghrelin, a peptide secreted by the stomach, stimulates appetite and hunger (Meier and Gressner,

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2004), increases before meals, and correlates with hunger in humans (Arvat et al., 2000; Muccioli et al., 2002). Sleep processes are also related to ghrelin (Steiger, 2007). A number of studies have shown that ghrelin levels rise at night (Cummings et al., 2001,2002; Dzaja et al., 2004; Koutkia et al., 2004), although some have not (Barkan et al., 2003; Natalucci et al., 2005). Experimental sleep deprivation blunts nighttime ghrelin levels (Dzaja et al., 2004) yet leads to increases in daytime levels in healthy humans (Spiegel et al., 2004). Conversely, ghrelin administration increases non-REM sleep in humans (Kluge et al., 2008).

Leptin is secreted primarily by adipocytes and signals the hypothalamus regarding the degree of fat stores in the body; decreased leptin indicates caloric shortage, whereas increased levels promote energy expenditure. In contrast with ghrelin, sleep deprivation decreases daytime leptin amplitude and levels in healthy human volunteers (Mullington et al., 2003; Spiegel et al., 2004).

In this study, we evaluated the relationships between sleep and nocturnal levels of ghrelin and leptin in chronic insomniacs. Although no study to date has examined these hormones in insomnia patients, based on experimental sleep deprivation studies with healthy sleepers (Mullington et al., 2003; Dzaja et al., 2004), we hypothesized that nocturnal levels of ghrelin and leptin would be decreased in men diagnosed with chronic insomnia as compared to age-, gender- and body weight-matched controls.

2. Methods

2.1. Subjects

Thirty-eight men participated in this study, divided into two groups, those with primary insomnia ($n = 14$) and healthy controls ($n = 24$). Subjects gave informed consent under University of California, San Diego (UCSD) Protocol No. 96095. Control subjects were recruited by the UCSD Mental Health Clinical Research Center (MHCRC) via community outreach lectures and newspaper advertisements. Controls selected for entry into the study were age- ($\pm$5 years) and body weight-matched ($\pm$2.6 kg/5 pound) with insomnia patients. Insomniac subjects responded to advertisements that solicited individuals with sleep complaints.

Prospective subjects underwent a telephone screening, and those eligible subjects were invited for an in-person assessment. During this session, individuals were informed about the study, gave informed consent, and underwent a comprehensive psychiatric and medical evaluation by a MHCRC psychiatric fellow-physician. Subjects received a structured sleep disorders interview and the structured clinical interview for DSM-III-R/DSM-IV (SCID). They provided a medical history and an interviewing physician conducted a review of systems, and physical examination. Subjects had their blood drawn for chemistry panel, complete blood cell count, and thyroid tests and HIV test.

Psychiatric diagnoses were confirmed via a consensus meeting of MHCRC psychiatrists, research fellows, and nursing staff. Control subjects were required to have no current or lifetime history of sleep, mood, anxiety or psychotic disorders as obtained via SCID assessment. Insomnia patients had to meet DSM-IV criteria for primary insomnia; i.e., subjects reported insomnia lasting at least 1 month, with significant distress or impairment and in the absence of any other current sleep, mood, anxiety, psychotic, substance use or eating disorder or medical condition. In addition, insomnia patients were given a sleep diary to complete for 2 weeks prior to their laboratory sleep assessment to confirm that they had difficulty initiating or maintaining sleep on at least 4 nights per week. The diaries also confirmed that subjects had a habitual sleep–wake pattern that would be similar to the laboratory sleep assessment protocol.

All subjects reported being in good medical health. No subject reported any acute infection, or a chronic medical condition such as diabetes, cancer, or chronic obstructive pulmonary disease. Eligible insomnia patients did not report current use of psychotropic medications; two insomniacs reported use of a benzodiazepine 7 and 14 days prior to study. All subjects had chemistry panel, complete blood cell count, and thyroid tests that were within normal limits and all subjects were tested negative for HIV antibodies.

2.2. Procedures

Laboratory sleep assessment included two nights of polysomnography. The timing of when lights were turned off (''lights out'') was based on self-reported lights out time from their sleep diary. Lights out times ranged between 2200 h and 2400 h. During the first night, subjects had an opportunity to get acclimated to the laboratory and EEG electrodes. In addition, recordings of pulse oximetry for oxygen desaturation were obtained to exclude subjects with sleep apnea (>10 apneas or hypopneas per hour of sleep). During the second night, polysomnography assessment included placement of electrodes for EEG (C3 or C4), electrooculography, and submental electromyography recordings. EEG sleep records were visually scored according to the criteria of Rechtschaffen and Kales (1968).

At 2100 h, a nurse inserted an intravenous catheter into the non-dominant vein of the subject, from which time the subject remained supine. To minimize disturbing subjects' sleep, blood was drawn periodically from the catheter via a long thin plastic tube, every 30 min starting at 2200 h until 0600 h. For the current study, assays were done on blood collected at 2300 h, 0200 h and 0600 h. Samples were immediately placed on ice, centrifuged at 4 °C, and stored at −80 °C until assay.

2.3. Leptin and ghrelin assays

Plasma leptin and total ghrelin levels were measured by competitive radioimmunoassay using commercial RIA kits (Cat# HL-81K and GHRT-89HK respectively) from Linco Research (now part of Millipore), St. Charles, MO. The assays were run on Titertek's Auto Gamma Counter (AGC 4/600 Plus) using PROCOMM plus software. Data reduction (5-PL fit curve) and calculation of results were performed using STATLIA 3.2 software (Brendan Technologies, Carlsbad, CA). Lower limit of detection (LOD) for leptin was 0.5 ng/mL and for ghrelin 96 pg/mL. Intra- and inter-assay precisions for leptin were 3.4–8.35% and 3.0–6.2% and for ghrelin were 5.6–8.7% and 7.9–11.7% respectively.

Table 1 Characteristics of controls and insomnia patients.

	Healthy controls $n = 24$	Insomnia patients $n = 14$	F	p
Age (year)	45 ± 12	49 ± 14	1.0	.32
Weight (kg)	80.73 ± 8.6	82.1 ± 10.9	.22	.64
Education level (year)	16 ± 2	16 ± 2	.01	.98
			χ^2	p
Ethnicity (% European American)	75%	71%	3.9	.27

2.4. Statistical analyses

Group differences in demographic and sleep measures were tested using a one-way analysis of variance (ANOVA). For leptin and ghrelin, a series of 2×3 repeated measures ANOVA were run, with a group status (control vs. insomnia) by time (2300 h, 0200 h and 0600 h) serving as the independent variables and leptin and ghrelin as the dependent variables.

3. Results

3.1. Demographic measures

Demographic data are presented in Table 1; men diagnosed with primary insomnia and healthy control males did not differ on age, body weight or education level. Ethnic composition of the groups was similar as well.

3.2. Sleep measures

Measures of sleep continuity and architecture are presented in Table 2. Insomnia patients had significantly less total sleep time and poorer sleep efficiency than healthy controls (p's $< .01$). The groups had similar sleep onset times, but insomnia patients evidenced a trend ($p = .06$) for higher wake after sleep onset (WASO). Sleep architecture also differed between the groups; insomnia patients spent significantly less time in stage 2 and REM sleep and more time in stage 1 sleep than healthy controls (p's $< .05$). Both groups had similar amounts of stages 3 and 4 sleep.

3.3. Leptin and ghrelin levels

As illustrated in Fig. 1, ghrelin levels across the night were significantly different between the groups, $F(1, 36) = 191$, $p < .0001$, with insomnia patients having lower ghrelin than healthy controls. Follow-up ANOVA tests at each time point indicated that insomnia patients had lower ghrelin at 2300 h ($p < .01$), 0200 h ($p = .08$), and 0600 h ($p < .01$). There was also a significant overall time effect for ghrelin, $F(2, 72) = 7.7$, $p < .001$, with levels at 0200 h being higher than those at 2300 h ($p = .05$) and at 0600 h ($p < .01$).

Leptin levels across the night were similar in both groups ($p > .5$). However, there was a significant overall time effect, $F(2, 72) = 7.3$, $p < .001$. Leptin levels at 0200 h were significantly higher than levels at 2300 h and 0600 h (p's $< .01$; Fig. 1). The pattern of findings for ghrelin and leptin remained the same with the removal of two insomnia patients who had reported benzodiazepine use 7 and 14 days earlier.

3.4. Associations between leptin, ghrelin and sleep measures

To further evaluate the association between sleep and nocturnal levels of ghrelin and leptin, a series of Spearman's correlations were run comparing total sleep time, sleep efficiency, stages 1 and 2, slow wave sleep, REM sleep with levels of leptin and ghrelin. Stage 1 sleep negatively correlated with ghrelin at 2300 h ($\rho = -0.28$, $p = 0.07$), at 200 h ($\rho = -0.30$, $p = 0.05$), and at 600 h ($\rho = -0.41$, $p = 0.007$) in the total sample. Other correlations were not statistically significant.

4. Discussion

Insomnia patients had significantly lower nocturnal ghrelin levels measured at three times (2300 h, 0200 h and 0600 h) than age- and weight-matched healthy control subjects. Leptin levels across the night were similar in both groups.

Table 2 Sleep characteristics of healthy control and insomnia patients.

	Healthy controls ($n = 24$)	Insomnia patients ($n = 14$)	F	p
Total sleep time (min)	393 ± 41	344 ± 68	7.8	.008
Total sleep efficiency (%)	89 ± 5	81 ± 14	7.8	.008
Sleep onset (min)	10 ± 7	29 ± 69	1.9	.18
WASO (min)	36 ± 21	52 ± 27	3.9	.06
Stage 1 (min)	25 ± 12	35 ± 19	4.5	.04
Stage 2 (min)	242 ± 32	200 ± 51	9.8	.003
Stage 3 (min)	27 ± 18	23 ± 16	.3	.6
Stage 4 (min)	12 ± 17	21 ± 28	1.4	.2
REM (min)	84 ± 24	64 ± 22	6.0	.02

WASO = waking after sleep onset; REM = rapid eye movement. Values for controls and insomnia patients represent mean $\pm$ S.D.

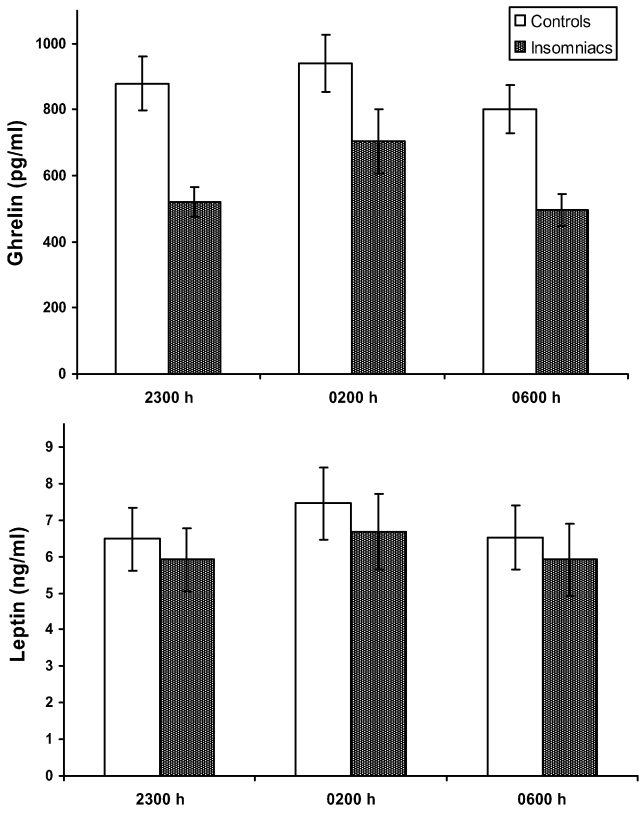

Fig. 1 The figure depicts nighttime circulating levels of ghrelin and leptin in insomnia patients and healthy controls. Ghrelin levels were consistently lower in insomnia patients vs. healthy controls, $F(1, 36) = 191$, $p < .0001$. Follow-up ANOVA tests at each time point indicated that insomnia patients had lower ghrelin at 2300 h ($p < .01$), 0200 h ($p = .08$), and 0600 h ($p < .01$). There was a significant time effect as well, $F(2, 72) = 7.7$, $p < .001$, with levels at 0200 h being significantly higher than levels at 2300 h ($p = .05$) and at 0600 h ($p < .01$). Leptin levels were similar in both groups and there was a significant time effect, $F(2, 72) = 7.3$, $p < .001$, such that levels at 0200 h were higher than levels at 2300 h and 0600 h (p's $< .01$). Bars denote standard error.

Insomnia patients, who were diagnosed based on clinical interviews using DSM-IV criteria, also had significantly poorer sleep as indicated by polysomnography including less total sleep time, less time spent in stage 2 and REM sleep, more time in stage 1 sleep and poorer sleep efficiency, as compared to healthy controls. Consistent with previous findings (Schuessler et al., 2005), ghrelin levels negatively correlated with amount of stage 1 sleep. Ghrelin levels did not correlate with any other sleep measure.

Ghrelin plays a role in both energy intake and sleep processes. Ghrelin stimulates appetitive behaviors; levels rise before mealtime and daytime administration promotes appetite and food intake (Cummings and Shannon, 2003). At night, recent evidence suggests that ghrelin affects sleep. Ghrelin levels rise between 0100 h and 0300 h during sleep and ghrelin stimulates the nocturnal rise in growth hormone. Intravenous administration of ghrelin before bedtime can increase non-REM sleep in men (Kluge et al., 2008), although sleep propensity and the timing of administration are important. Ghrelin administered in the very early morning (after 400 h) did not affect sleep (Kluge et al., 2007b). Sleep processes can exert effects on ghrelin levels. Experimental sleep deprivation in healthy adults is associated with *decreased* ghrelin levels at night (Dzaja et al., 2004) but *increased* levels during the following day, especially in the following afternoon (Spiegel et al., 2004) and evening (Schussler et al., 2006). Similarly, in a community-dwelling sample of men and women from the Wisconsin Sleep Cohort Study, increased morning levels of ghrelin were associated with less sleep time (Taheri et al., 2004). Findings from the current study are consistent with experimental sleep deprivation in which nocturnal ghrelin levels were also found to decrease (Dzaja et al., 2004). In the current study, insomnia patients had ghrelin levels that were approximately 30% lower than healthy controls, a difference similar in magnitude to those found when comparing obese (Tschop et al., 2001) or type 2 diabetic adults (Poykko et al., 2003) with healthy controls.

Leptin levels were higher at 200 h than the other two time points in both groups; however, across the night, leptin levels did not differ between the groups. Like ghrelin and leptin levels rise shortly after sleep onset and this increase is blunted during experimental sleep deprivation (Mullington et al., 2003). Although mean values for leptin were lower in insomnia patients, these values were not significantly different from healthy controls.

Decreased nocturnal ghrelin in insomnia may be driven by the autonomic nervous system (ANS). Decreases in efferent vagal activity and/or blockade of vagal signaling are associated with decreased ghrelin expression (Williams et al., 2003; Broglio et al., 2004; Maier et al., 2004). Insomnia patients have decreased efferent vagal activity, as measured by decreased heart rate variability (Bonnet and Arand, 1998), along with heightened sympathetic activity, evidenced by increased circulating catecholamines (Irwin et al., 2003). However, the effects of sympathetic activity on ghrelin are less clear; changes in circulating epinephrine do not affect ghrelin levels, yet short-term sympathetic nerve stimulation boosts ghrelin expression (Mundinger et al., 2006). Similarly, sympathetic response to short-term psychological stress is not associated with ghrelin responsivity (Rouach et al., 2007).

The findings from the current study suggest an underlying disruption in ghrelin expression that may be important not just for sleep but also for energy balance. Although the current study did not address weight gain, our findings suggest that ghrelin may be important to examine as a mediator or moderator relating poor sleep to body weight. Chronic insomnia is prospectively associated with weight gain (Janson et al., 2001; Hasler et al., 2004) and is cross-sectionally associated with obesity (Foley et al., 1995; Taheri et al., 2004). Future prospective studies are needed to examine whether ghrelin mediates the relationship between poor sleep and weight gain in insomnia patients.

This study had a number of limitations. The sample was small and comprised entirely of men; the nocturnal profile of leptin and ghrelin in female insomnia patients is unknown. Recent work suggests that gender plays an important role in regulating energy balance with sleep processes (Schuessler et al., 2005). In one study with only women participants,

ghrelin administration provoked the expected increase in growth hormone, but no changes in sleep parameters (Kluge et al., 2007a). Thus larger studies incorporating male and female insomnia patients are needed. In addition, the number of nocturnal time points was limited in this study and future studies that incorporate more frequent blood draws both at night and during the day are needed. Future work may also elucidate the complex interactions between the course of insomnia, behavior, mood and food intake with energy balance. Lastly, it is unclear whether these findings reflect state or trait changes in hormone regulation. Future work examining ghrelin and leptin and treatment of insomnia (e.g. medication management or behavior therapy) may help determine whether energy balance regulation in insomnia patients can be adjusted with treatment.

Although the number of studies examining ghrelin and leptin continues to increase dramatically, the mechanisms as to how these hormones coordinate both appetitive drives and sleep processes are unclear. Decreased ghrelin in insomnia patients reflects altered endocrine energy balance and indicates that in addition to short-term, experimental sleep loss, long-term sleep difficulties are also associated with altered ghrelin expression.

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All authors declare that they have no conflicts of interest.

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