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Dim light melatonin onset in alcohol-dependent men and women compared to healthy controls

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Abstract

Background—Sleep disturbances in alcohol-dependent (AD) individuals may persist despite abstinence from alcohol and can influence the course of disorder. Although the mechanisms for their sleep disturbances are not well understood and some evidence suggests dysregulation of circadian rhythms, dim-light melatonin onset (DLMO) has not previously been assessed in AD vs. healthy control (HC) individuals in a sample that varied by sex and race.

Methods—Fifty-two AD participants (mean age 36.0 ± 11.0 years, 10 women) who were 3–12 weeks since their last drink (mean abstinence 57.9 ± 19.3 days) and 19 age- and sex-matched HCs (mean age 34.4 ± 10.6 years, 5 women) participated. Following a 23:00 – 06:00 h at-home sleep schedule for at least 5 days, and screening/baseline nights in the sleep laboratory, participants underwent a 3-hr extension of wakefulness (02:00 h bedtime) during which salivary melatonin samples were collected every 30 minutes beginning at 19:30 h. The time of DLMO was the primary measure of circadian physiology and was assessed with two commonly used methodologies.

Results—There was a slower rate of rise and a lower maximal amplitude in the AD group. DLMO varied by methodology used. Using 3 pg/ml as a threshold, no significant differences between the AD and HC groups were found. Using two standard deviations above the mean of the first 3 samples, AD DLMO occurred later 21:02 (SD=0:41) than HC 20:44 (SD=0:21) t=-2.4, (p=.02).

Conclusions—While melatonin in the AD group appears to have a slower rate of rise, using well-established criteria to assess salivary DLMO did not reveal differences between AD and HC participants. Only when capturing melatonin when it is already rising was DLMO significantly delayed by a mean 18 min in ADs. Future circadian analyses on alcoholics should account for these methodological caveats

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Declaration of interest

Dr. Armitage was a paid consultant for Eisai Inc during the study, but unrelated to the present research. The other authors have indicated no financial conflicts of interest.

Keywords

Alcohol; Melatonin; Men; Women; Sleep; Dim light melatonin onset (DLMO)

Introduction

It is well known that sleep complaints characterize protracted withdrawal from alcohol dependence and have been associated with relapse (Brower 2001, 2003; Brower et al. 2001). During both drinking periods and withdrawal, alcohol-dependent (AD) individuals commonly experience problems falling asleep and decreased total sleep time. However, the specific mechanisms of these sleep disturbances are not well understood. Possible mechanisms, after excluding other causes such as co-occurring mental and physical disorders, include impairments in either sleep homeostasis or circadian rhythm regulation.

A number of studies demonstrate that alcohol can interfere with the endogenous circadian pacemaker over time (Hasler et al. 2011). Acute ethanol exposure can block photic and non-photic circadian phase resetting. Chronic alcohol exposure, during postnatal development, also affects the phase-shifting response of the suprachiasmatic nucleus (SCN) to bright light(Farnell et al. 2004; Ruby et al. 2009a; Ruby et al. 2009b); resulting in dysregulation of neurotrophic factors involved in mood, reward, and sleep regulation (Allen et al. 2004), and alters the expression of *Per1*, *Per2* and *Per3* genes in the SCN (Chen et al. 2004) in rats. It is unclear whether this dysregulation affects drinking behaviour. However, one preclinical study showed a reduction in ethanol drinking in rats following repeated light-dark phase shifts (Rosenwasser et al. 2010).

Mixed results have been reported in clinical studies of alcohol administration to healthy participants without alcohol dependence. Some studies found that administering alcohol prior to bedtime reduced melatonin levels (Ekman et al. 1993; Rupp et al. 2007) with higher doses of alcohol resulting in greater reductions (Rojdmark et al. 1993). Other studies reported no differences in melatonin levels following alcohol administration (Danel and Touitou 2006; Plenzler et al. 1996). Similarly, when alcohol was administered to healthy volunteers over a 24-hour period, the overall circadian phase of melatonin was not affected (although it was delayed in 6 out of 11 subjects) and there was no daytime melatonin secretion, suggesting that the effects of alcohol on sleep may not be due to its direct effect on the circadian system(Danel and Touitou 2006). Unlike healthy participants exposed to acute doses of alcohol, however, AD individuals generally have prolonged periods of exposure to intoxicating doses of alcohol.

In AD participants, melatonin levels have been found to be higher during active drinking compared to two weeks of abstinence (Fonzi et al. 1994; Murialdo et al. 1991). Another study showed that during an episode of drinking, melatonin was actually higher during the day (8 a.m.-11 p.m.) than at night (11 p.m.-8 a.m.), opposite of what is found in healthy young adults. After 15 days of withdrawal, the day to night melatonin rhythm normalized (higher levels at night) in 4 of the 7 AD patients (Danel et al. 2009). It has also been established that misalignment between the midsleep of the sleep wake cycle and DLMO has been associated with depression ratings (Lewy et al. 2006), suggesting that there may be

similar associations in alcoholism, given the high comorbidity between depression and alcoholism. For example, winter depressive patients are delayed with respect to normal controls (Lewy et al., 1987, 1998b; Sack et al., 1990).

One study (Kuhlwein et al. 2003), showed lower levels of plasma melatonin in the early part of the night in African American AD men, sober for 26 days, compared to healthy control (HC) men. They reported no differences in melatonin in the later part of the night. In addition, there was a significant 90-minute delay in the melatonin plateau in the AD vs. HC men.

A major limitation of these studies is that they included few women and there was limited information about the racial make-up of the sample, (Danel et al. 2009; Rupp et al. 2007) except for Kühlwein 2003 (Kuhlwein et al. 2003) in which the entire sample was African American, albeit all men. Of the four studies listed above involving AD participants, (Danel et al. 2009; Fonzi et al. 1994; Kuhlwein et al. 2003; Murialdo et al. 1991) there was only one female participant(Danel et al. 2009) enrolled. Therefore, it is unclear to what degree circadian phase or amplitude of melatonin may differ by sex or race in AD individuals. When assessing racial differences in endogenous period, healthy African American participants had larger phase advances and smaller phase delays in response to a light stimulus(Smith et al. 2009).

The current study compared DLMO and related melatonin parameters in a sample of AD and HC participants that included men and women, and both African-Americans and Caucasians. We hypothesized that AD participants would have delayed DLMO times, lower melatonin levels in the first half of the night, and a delayed nocturnal maximum of melatonin during the sampling period under dim light conditions.

Methods

Participants

Participants were recruited through a combination of advertisements, posted flyers in affiliated clinics, and either clinician- or self-referral. A total of 73 participants underwent the study procedures. One participant was excluded because of a positive drug screen on the study night; one participant was excluded because 7 out of 14 melatonin samples were missing. Therefore, data for a total of 71 individuals were included in these analyses, 52 AD participants (mean age 36.0 ± 11.0 years, 10 women) (who were 3–12 weeks since their last drink and 19 age- and sex-matched HCs (mean age 34.4 ± 10.6 years, 5 women) See Table 1. AD participants met past-year DSM-IV diagnostic criteria for alcohol dependence as determined by the Structured Clinical Interview for DSM-IV Diagnoses (First et al. 1997). ADs were excluded if they met DSM-IV criteria for dependence on any other substance except nicotine; if they met past one-month criteria for any mood disorder, anxiety disorder, or eating disorder; had a lifetime diagnosis of bipolar disorder or any psychotic disorder; or had any medical illness or took medications, including all psychiatric medications, known to affect sleep.

This study was approved by the University of Michigan Medical Institutional Review Board and written informed consent was obtained from each participant prior to starting the study. The protocol conformed to international ethical standards (Portaluppi et al. 2010).

Baseline sleep and drinking measures

Pittsburgh Sleep Quality Index (PSQI)(Buysee et al. 1989)—The PSQI is a 19-item self-administered questionnaire consisting of seven components (sleep perception, sleep latency, sleep duration, sleep efficiency, sleep disorders, hypnotic use, and daytime sleepiness) weighted from 0 to 3. Overall scores range from 0–21 with lower scores indicating better sleep. A score greater than five indicates a clinically significant sleep problem involving at least two components.

Insomnia Severity Index (ISI)(Morin 1993)—The ISI is a 7-item self-administered questionnaire designed to assess the nature, severity, and impact of insomnia over the past week. All items are rated on a 5-point Likert scale (0=not at all to 4= extremely) yielding a score from 0–28. Scores from 0–7 indicate absence of insomnia, 8–14 indicate sub-threshold insomnia, 15–21 indicate moderate insomnia, and 22–28 indicate severe insomnia.

The Obsessive Compulsive Drinking Scale (OCDS)(Anton et al. 1996)—A 14-item self-administered questionnaire that measures urges or craving to drink alcohol over the past 2 weeks. A higher score indicates greater urges to drink.

The Short Inventory of Problems (SIP; a.k.a. the Short Index of Problems) (Feinn et al. 2003)—A 15-item, self-administered questionnaire that asks about drinking-related problems during the past 4 weeks. Scores range from 0 to 45 with higher scores indicating greater problems.

Severity of Alcohol Dependence Questionnaire (SADQ)(Stockwell and Murphy 1983)—This self-administered questionnaire to assess severity of the disorder was administered only to the AD participants. In order to quantitate their alcohol consumption prior to study entry, we used four questions from this questionnaire pertaining to the amount of drinking during a recent period of heavy drinking prior to entering the study. Each question was followed by a 4-point Likert scale ranging from 0=almost never to 3=nearly always. These questions were: 1) During a heavy drinking period, I drink more than 6 drinks per day, 2) During a heavy drinking period I drink more than 12 drinks per day, 3) During a heavy drinking period, I drink more than 24 drinks per day, and 4) During a heavy drinking period, I drink more than 48 drinks per day. Equivalencies between different types of alcohol were listed on the form immediately prior to these questions (e.g., 1 bottle of wine (750 ml) = 6 beers = 6 shots of liquor = 6 drinks). The total score ranged from 0 to 12 with higher scores indicating heavier drinking.

Sleep Schedule

Participants were instructed to keep a regular 23:00 to 06:00 h sleep schedule for at least five days before laboratory assessment. Information regarding habitual sleep schedule prior to the study's recommended sleep schedule was obtained by relevant questions in the PSQI.

Adherence to the regular sleep schedule at home was verified via actigraphy and daily sleep diaries (Table 1). AD participants were studied in the sleep laboratory 58.0 ± 19.4 (range=31–100) days after their last drink (54 days in females; 59 days in males) as determined by the time-line follow-back interview (Maisto et al. 1979; Sobell et al. 1979; Sobell et al. 1988) and breath testing on at least 4 occasions during the screening/baseline period, including the night of the study. Urine drug screens were also negative on each of these occasions.

DLMO Assessment

Salivary melatonin was collected at 30-minute intervals between 19:30 h and 02:00 h for a total of 14 samples, while participants were seated in their sleep laboratory recording rooms, illuminated by dim (<40 lux) incandescent light, during their third night in the sleep laboratory. Subjects rinsed their mouth with water and brushed their teeth, without toothpaste, 15 minutes prior to the collection of each sample. Samples were collected using Salivette tubes (Sarstedt, USA). Subjects were instructed to chew on a sterile non-impregnated cotton swab for 2 min and return it to the Salivette tube. Tubes were centrifuged at 3,000 rpm for 10 min at room temperature within 2 hours of collection, and stored at −20°C until analyzed by the Core Assay Facility at the University of Michigan. Samples containing 1 ml of saliva were assayed in duplicate using radioimmunoassay kits, which could each analyze up to 200 samples (American Laboratory Products Company Ltd., Windham, NH). The range of possible values was between 0.5 - 50 pg/ml. The sensitivity of the assay was 0.2 pg/ml. The inter-assay variability ranged from 16–22% for a high and 1.7 to 2.4% for low.

Calculation of DLMO

Dim-Light Melatonin Onset (DLMO) refers to the time at which melatonin levels reach a threshold value (MEL_threshold). DLMO was calculated according to two commonly used methodologies (Voultsios et al. 1997). We used a 3 pg/mL threshold **(Method 1)** for salivary melatonin because it has been established in the literature as effectively capturing the inflection point of melatonin secretion in most individuals. It was also used as our primary method of analysis because melatonin concentrations analyzed by other methods revealed that melatonin was already rising. We used a second method, mean plus two standard deviations above the mean of the first three samples **(Method 2),** because it was established for individuals with lower secretion levels and to control for inter-individual differences in amplitude. Moreover, Voultsios et al (1997) described this method to be "more successful and statistically superior" to other methods (p. 463). The first step in calculating when this threshold is reached (i.e., time of DLMO) is finding the time window within which it would have occurred. Because melatonin samples were collected every 30 min, the exact time of DLMO always occurred within a 30-min time window. The melatonin concentrations at those times were directly measured and can be represented by the variable names, MEL_preDLMO and MEL_postDLMO, respectively. For example, if the calculated value for MEL_threshold was 1.90 pg/ml, and the measured melatonin concentrations at 20:00 h and 20:30 h were 1.70 pg/ml and 1.95 pg/ml, respectively, then the time window for the MEL_threshold was between 20:00 h, and 20:30 h. In this example, 20:00 h = preDLMO time and 20:30 h = postDLMO time, and DLMO falls between those times. The amount of

time in min between DLMO and postDLMO (i.e., postDLMO – DLMO) can be calculated as a proportion of melatonin concentrations, multiplied by the time window (30 min), using the following formula:

> postDLMO – DLMO = (MEL_postDLMO - MEL_threshold)/(MEL_postDLMO - MEL_preDLMO) * 30 min.

Substituting the melatonin concentration values for the example into this formula would yield a time of 6 min. By subtracting 6 min from the postDLMO time of 20:30 h, DLMO would be calculated as 20:24 h.

Phase angle difference (PAD), the difference between a sleep marker and circadian marker, was calculated and shown in Table 2. We chose to calculate the difference between a subjective measure, midpoint of sleep according to sleep diary per (Lewy et al. 2006) and DLMO as determined by each of the two methods. The time course of melatonin levels across the sampling period was also analysed both for diagnostic group differences and to determine when during the night those differences may have occurred.

Statistical Analyses

Independent samples t-tests and chi square tests were conducted to compare diagnostic groups on demographic and questionnaire data. To analyse for differences between diagnostic groups in DLMO, independent samples t-tests were also used. To calculate the melatonin profile across the night, a between subjects repeated measures ANOVA was used. Pearson correlational analyses were conducted to asses statistical relationships between variables. All statistical analyses were two-tailed, used a p value of 0.05 for significance, and were performed by using SPSS version 17.0.

Results

Clinical variables

Prior to adhering to the study's 23:00-06:00 sleep schedule, the PSQI revealed that AD participants usually went to bed at 23:43 (1:24) and the HCs went to bed at 23:41 (0:53) (p=NS). The typical sleep onset latency was 33.3 min (22.5) in the ADs and 12.0 min (7.1) in the HCs (t=6.2, p<.01). The usual wake time for the ADs was 07:01 (1:46) and 07:36 (0:58) in the HCs (p=NS).

Baseline demographic and questionnaire data are presented by diagnostic group in Table 1. HCs had more years of education, 15.6 years (1.9) than the AD group, 13.2 years (2.0). AD participants reported significantly higher ISI, PSQI, OCDS, and SIP scores than HCs. All participants maintained approximately a 23:00 h to 06:00 h sleep schedule and sleep variables did not differ between diagnostic groups (Table 1).

Melatonin profile across the night

Figure 1 illustrates a slower rate of rise and a lower maximal amplitude in the AD group. There was no time by diagnosis interaction, [F (13, 48)=1.1, p=.39], but there was a trend towards a between subjects effect for diagnosis [F (1, 60)=2.9, p=.09]. There was a

significant main effect for time on melatonin concentrations across the night [F (13, 48) =7.8, p<.005].

To test the hypothesis that difference between diagnostic groups would be seen in the first part of the night, further analyses were conducted to determine when during the night the groups differed significantly in melatonin levels (Fig. 1). Medium to large effect sizes were detected at 22:00 [AD 4.8 (3.7) vs. HC 8.2 (8.5) t=1.5, p=.15, Cohen's d = −0.52; 22:30 [AD 5.6 (4.2) vs. HC 8.4 (7.0) t= 1.5, p=.16 Cohen's d = −0.49]; 23:00 [AD 6.5 (4.8) vs. HC 9.3 (6.9), t=1.8; p=.08, Cohen's d = -.47]; and 23:30 [AD 6.8 (4.7) vs. HC 9.3 (6.0), t=1.6, p=.16, Cohen's d = −0.46]. To illustrate the size of the effect, melatonin levels were summed across the night for each group and the mean sums were compared, showing a trend towards a difference between the AD [73.1 (44.1) pg/ml] and HC [102 (71.7) pg/ml] groups with a medium effect size (Cohen's d = .49). Finally, we did not find delayed maximal melatonin concentrations during the sampling period for AD (01:00) vs. HC (01:30) participants (p=ns).

Melatonin variables

DLMO was analysed according to two commonly used methodologies. Method 1 did not yield any significant difference between DLMO between diagnostic groups (Table 2). When using Method 2, DLMO occurred significantly later in the AD participants [21:02 (0:41) h] than in the HCs [20:44 (0:21) h]. When analyses were conducted for Caucasians only and for males only, results were similar across methodologies (significant for Method 2 only). HCs also had a larger phase angle difference between DLMO and the middle of the habitual sleep period reported in the diary (see Table 2).

DLMO and drinking prior to study

Pearson correlation analysis was conducted between the sum of the four items inquiring about quantity of drinking on the SADQ and DLMO to explore whether amount of prior drinking was associated with DLMO. There was an inverse relationship between DLMO Method 1 and amount of drinking (r=-.28 p=.049), i.e., the more drinking the *earlier* the time of DLMO. There were no associations between drinking severity and DLMO Method 2. Pearson correlations were conducted between days sober prior to the sleep laboratory night and DLMO from both methods. Neither correlation was statistically significant.

Discussion

This study found that while there appears to be a slower rate of rise and a lower maximal amplitude in the AD group, few other differences in the melatonin profile between AD and HC participants were found. Using a standard 3 pg/ml as the DLMO threshold, there were no significant differences between groups. Only when using a different criterion, the mean plus 2 standard deviations, was DLMO in AD participants significantly delayed compared to HC. The AD group showed an 18 minute delay in DLMO.

To our knowledge, this is the first study of DLMO in AD individuals that included both men and women as well as Caucasians and African Americans. The DLMO results with Method 2 are consistent with those of Kulhlwein et al (2003) (Kuhlwein et al. 2003) who found that

the time of plateau in nocturnal melatonin levels was phase delayed in their AD group, although by 90 min. However, this result was not obtained with the other DLMO calculation method. Moreover, their sample was limited to African American men. Our result, at least with Method 2, remained significant when African American men were excluded from the analysis, thus providing very tentative evidence that white males with alcohol dependence may also have delayed circadian phase. Also consistent with the findings of Kuhlwein et al (2003), AD participants had lower melatonin levels in the first half of the night (22:00 to 23:30 hours) than HCs. Whether these potential differences in circadian physiology precede the onset of alcohol dependence or results from the toxic effects of chronic heavy drinking is unknown and a question for future investigation.

Our finding that DLMO by Method 1 was correlated with amount of drinking prior to the study suggests that perhaps there is a direct pathophysiological link between alcohol and circadian function, extending preclinical studies in mice that showed that forced ethanol consumption affects circadian activity in a dose-dependent manner (Brager et al. 2010, 2011). Brager et al 2011 also found that acute alcohol consumption inhibits the phase resetting response of the mouse circadian clock to non-photic (serotonergic) phase resetting properties.

Our results should be interpreted in the context of the study's limitations. First, in addition to the small number of women, there were too few participants of African American and Hispanic heritage to analyse them separately. We were able to analyse Caucasians separately to show a parallel between our delayed DLMO results and previously published results of delayed peak melatonin levels in African Americans (Kuhlwein et al. 2003). No such parallels, however, were possible for Hispanic individuals.

Second, participants kept a 7-h, 23:00-06:00 h fixed schedule to control for the influences of prior wakefulness and the light-dark cycle. This may have influenced our results in two significant ways: 1) This fixed sleep schedule prior to assessing DLMO may have obscured large phase differences between individuals and may have accounted for the lack of differences in DLMO between diagnostic groups using Method 1. DLMO-bedtime phase angle has been reported to range from −.30 hours (DLMO occurred after bedtime) to 5.79 hours. Many studies now start collecting samples 5–6 hours before bedtime to account for this (Burgess and Fogg 2008). DLMO has been found to be more highly correlated with sleep times in individuals who are free to sleep on their own schedule compared to those on a fixed sleep schedule (Burgess and Eastman 2005; Sletten et al. 2010). However, AD and HC male participants in the Kuhlwein study also maintained an approximately 23:00-06:00 hours fixed schedule and they found differences in melatonin between groups(Kuhlwein et al. 2003). 2) The enforced sleep timing prior to this study may have been unlikely to bring all the participants in line to a 2-hour DLMO-sleep onset phase angle. Moreover, given our hypothesis that there may be a delayed sleep phase in the participants, the 23:00-06:00 schedule may have been at an unnatural circadian phase for this group. The longer SOL in AD participants during baseline supports this presumption.

Third, our use of salivary melatonin assessment instead of plasma or serum may not have been sensitive enough to capture the subtle differences between groups. The higher

melatonin levels in plasma melatonin allow for greater sensitivity than urine or saliva, especially in those who have lower concentrations (Benloucif et al. 2008), such as in alcoholics. Previous studies examining the effects of alcohol ingestion on melatonin secretion have found differences analyzing blood samples, but no difference in urine (Ekman et al. 1993; Rojdmark et al. 1993) or saliva samples (Plenzler et al. 1996).

Finally, we used an established procedure (Lewy et al. 2006) to assess DLMO by obtaining evening samples of melatonin every 30 minutes. However, without an afternoon through overnight or 24-hour melatonin profile, we reduced the number of baseline samples prior to the inflection point in the melatonin profile. This may have resulted in melatonin levels that are rising above baseline levels in our DLMO calculation, therefore artificially delaying the DLMO in those individuals. It is worthy of note that Figure 1 does show that the melatonin concentration in AD participants appears to begin to decline at the 01:30 h time point.

Our results showed that a commonly used threshold for salivary DLMO (>3pg/ml) was not sufficient to detect differences in AD versus HC participants. Subtle differences only emerged when using a DLMO analysis method that captured melatonin levels when they had already begun to rise. Given that these study findings were highly dependent on the specific analyses used, future studies should collect melatonin for the entire night (i.e., a full 24-hours) in order to capture the melatonin profile. Not only will this allow more valid comparison of DLMO, this would also allow comparisons of total melatonin secretion, given that the current data suggest that overall melatonin levels, rather than timing of secretion, are what distinguish the two groups. Additional participants that vary by race and sex are also warranted in future studies to determine if differences in circadian physiology exist between ADs and HCs, or if these differences are restricted to subtypes.

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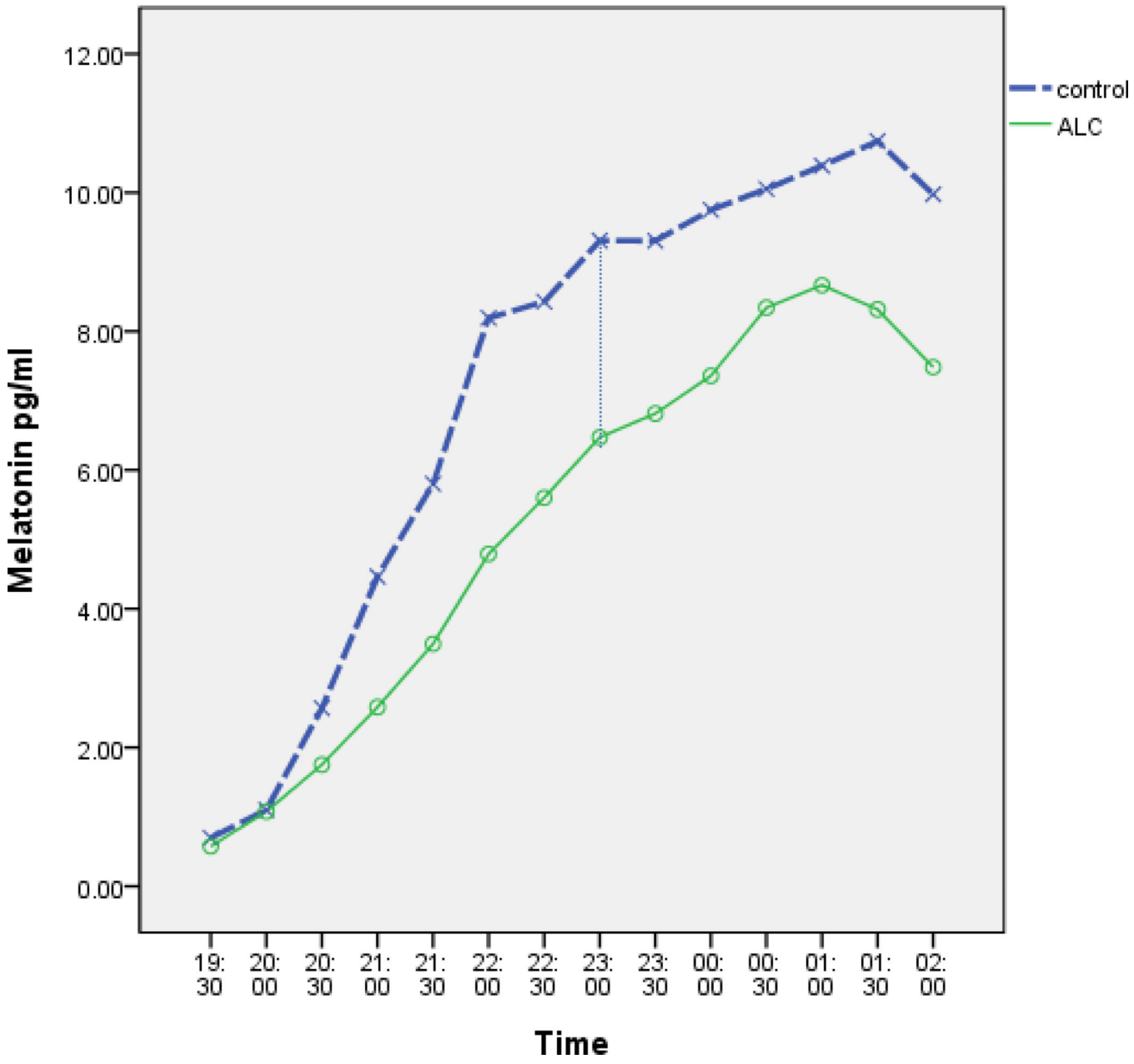

Figure 1. Melatonin profile in AD versus HC

Legend: Melatonin secretion in sampled across the night every 30 minutes from 19:30 to 02:00. Healthy control (HC) groupn= 19 are illustrated in dashed line with x data points (top line) and alcohol dependent (AD) group n=52 are illustrated in solid line with circle data points (bottom line). A thin vertical line at 23:00 indicates a trend ($p<.10$) towards a statistical difference between the profiles.

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Table 1

Means, standard deviations, and p values for demographic, sleep, and drinking variables by diagnosis.

	AD (n= 52)		**HC (n=19)**			
Demographics	M	SD	M	SD	T/chi	p
Age (y)	36.0	11.0	34.4	10.6	−.56	.58
Sex (male; female n)	42; 10		14; 5		**.41**	**.52**
Education (y)	**13.2**	**2.0**	**15.6**	**1.9**	**4.6**	**<.01**
Marital status (n)	**4 M; 48 NM;**		**7 M; 12 NM**		**11.7**	**.02**
Employment (n)	30 U; 22 E		7U; 12E		2.9	.23
Ethnicity (n)	**35C; 5H; 12AA**		**18C; 1AA**		**7.5**	**.03**
Sleep						
ISI	**9.2**	**5.8**	**1.5**	**1.6**	**−8.7**	**<.01**
PSQI	**7.1**	**3.7**	**2.6**	**1.3**	**−7.4**	**<.01**
Days sober	**57.9**	**19.3**	**NA**			
OCDS	**11.4**	**8.2**	**1.1**	**2.5**	**−7.5**	**<.01**
SIP	**14.0**	**12.1**	**0.3**	**1.1**	**−7.9**	**<.01**
SADQ	19.1	12.6	NA			
Sleep diary						
Bedtime (hh:mm)	22:59	00:17	23:04	00:11	1.2	.22
Waketime (hh:mm)	06:19	00:32	06:22	00:33	0.75	.46
SOL (min)	30.8	35.0	25.0	14.0	−.71	.48
WASO (min)	20.7	23.2	12.0	10.9	−1.6	.13
FNA	1.7	1.1	1.5	0.85	−.61	.54

Legend: AD=Alcohol dependent; HC=Healthy controls; NM=not married (includes never married, lives with partner, separated, and divorced); M=married; U=unemployed; E=employed; AA=African American; C=Caucasian; H=Hispanic; ISI=Insomnia Severity Index; PSQI=Pittsburgh Sleep Quality Index total score; Days sober indicates number of days sober before sleep laboratory night; OCDS=Obsessive Compulsive drinking scale; SIP=Short Inventory of Problems; SADQ=Severity of Alcohol Disorders Questionnaire. SOL= Sleep onset latency; WASO= Wake time after sleep onset; TST= Total Sleep Time; SE= Sleep efficiency; FNA=Frequency of nighttime awakenings.

Bold indicates significant difference in between diagnostic conditions. Chi-square likelihood ratio is reported here.

Table 2

Means, standard deviations, between group differences, and effect sizes for circadian variables.

	AD (n=52)		HC (n=19)				
	M	**SD**	**M**	**SD**	**t**	**p**	**Cohen's *d***
Melatonin characteristics							
DLMO (hh:mm) (Method 1)	22:02	1:24	22:06	1:21	−.21	.83	−0.05
DLMO (hh:mm) (Method 2)	**21:02**	**0:41**	**20:44**	**0:21**	**−2.4**	**.02**	**−0.55**
Sum (pg/ml)	73.1	44.1	102.0	71.7	1.6	.11	−0.49
Amplitude (pg/ml)*	9.7	6.6	11.8	8.2	1.7	.11	−.028
Meanconcentration across night (pg/ml)	5.3	3.2	7.4	5.2	1.0	.30	−.049
PAD Mid sleep (min) (Method 1)	270.6	83.4	281.2	82.8	.46	.65	−0.13
PAD Mid sleep (min) (Method 2)	**333.6**	**39.6**	**358.2**	**23.8**	**2.5**	**.02**	**−0.75**

Legend: AD=Alcohol dependent; HC=Healthy controls; DLMO=Dim light melatonin onset, Sum=sum of all melatonin concentrations for the sampling period; PAD=Phase angle difference between DLMO and mid sleep of sleep diaries; DLMO: Method 1=>3pg/ml; Method 2=mean plus 2 standard deviations above the mean of first three samples.

*Amplitude= the difference between the highest and lowest melatonin concentration for the sampling period.

Bold indicates significant difference in between diagnostic conditions.