Heavy alcohol use, rather than alcohol dependence, is associated with dysregulation of the hypothalamic–pituitary–adrenal axis and the autonomic nervous system

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Abstract

Background: Heavy alcohol use as well as alcohol dependence (AD) have been associated with dysregulation of the hypothalamic–pituitary–adrenal (HPA)-axis and the autonomic nervous system (ANS). However, the relative contribution of alcohol use and AD is unclear.

Methods: Baseline data were derived from 2947 persons of the Netherlands Study of Depression and Anxiety (NESDA), including non-drinkers (n = 498), moderate drinkers (n = 2112) and heavy drinkers (n = 337). We also distinguished between persons with no lifetime DSM-IV AD (n = 2496), remitted AD (1 year; n = 243), and current AD (<1 year; n = 208). ANS measures included ECG-based heart rate (HR), respiratory sinus arrhythmia (RSA, high RSA reflecting high cardiac parasympathetic control) and pre-ejection period (PEP, high PEP reflecting low cardiac sympathetic control). HPA-axis measures included the cortisol awakening response (area under the curve with respect to the ground [AUCg] and increase [AUCi]), evening cortisol and a 0.5 mg dexamethasone suppression test, all measured in saliva.

Results: Heavy drinkers showed higher basal cortisol levels (AUCg: p = .02; evening cortisol: p = .006) and increased cardiac sympathetic control (higher HR: p = .04; lower PEP: p = .04) compared to moderate drinkers. Persons with current or remitted AD did not differ from persons without lifetime AD on any of the HPA-axis or ANS indicators (all p > .33). Similar patterns of HPA-axis and ANS activity across alcohol use groups were found in persons with and without lifetime AD.

Conclusions: Our findings suggest that current heavy alcohol use, rather than current or remitted AD, is associated with hyperactivity of the HPA-axis and increased cardiac sympathetic control.

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1. Introduction

Heavy alcohol use and alcohol dependence (AD) have been associated with a wide range of physiological, psychological and behavioral problems. For example, heavy drinkers and persons with AD have an increased risk of cardiovascular disease (Corrao et al., 2000) and cancer (Bagnardi et al., 2001; Boffetta and Hashibe, 2006) as well as depressive and anxiety disorders (Burns and Teesson, 2000; Swendsen et al., 1998). In addition, alcohol use has been linked to harmful behavioral consequences such as aggressive behavior, crime and unintended injuries (Gmel and Rehm, 2003) and AD is characterized by a loss of control over the alcohol use (American Psychiatric Association, 2000). In an attempt to comprehend the common underlying mechanisms associating alcohol with these various problems, Thayer et al. (2006) proposed the neurovisceral integration model (Thayer and Lane, 2000), comprising the hypothalamic–pituitary–adrenal (HPA)-axis and the autonomic nervous system (ANS). They hypothesized that alcohol ingestion is directly associated with both stress systems, following which chronic activation, as in AD, may result in impaired inhibitory control (Thayer et al., 2006).

The HPA-axis has a key role in normal physiological processes and in adaptation to stress (Selye, 1936). It is responsive to the basic motivational processes, such as seeking food, ingestion of nutrients and threats to well being (Lovallo, 2006). Alcohol use stimulates the HPA-axis and, with that, causes stress-like cortisol responses resulting in elevated basal cortisol levels (Gianoulakis et al., 2003; Thayer et al., 2006) and an increased cortisol awakening response...
been linked to a blunted stress response for at least four weeks post-
use has been associated with hyperactivity of the HPA-axis, AD has
processes (Thayer and Lane, 2000). High parasympathetic con-
tory processes) and parasympathetic nervous system (inhibitory
flexible and adaptive behavior across challenging situations due
on alcohol were included.

Studies have also provided evidence for a role of the ANS in the
pathophysiology of alcohol use and AD. The ANS allows for
flexible and adaptive behavior across challenging situations due
to its interplay between the sympathetic nervous system (excita-
tory processes) and parasympathetic nervous system (inhibitory
processes) (Thayer and Lane, 2000). High parasympathetic con-
control is associated with adequate modulation of affect and emotion
and increased impulse control (Allen et al., 2000), whereas low
parasympathetic control is associated with affect dysregulation as
in persons with depressive and anxiety disorders (Thayer et al.,
1996, 1998). Previous studies have shown that alcohol use causes
increased sympathetic control (Ohiha et al., 2009; Ryan and Howes,
2002) and an acute reduction in parasympathetic control (Reed et
al., 1999; Vaschillo et al., 2008; Weise et al., 1986). Persons with AD
also showed a decrease in parasympathetic control and an increase
in sympathetic control during acute alcohol withdrawal and during
alcohol abstinence up to 4 weeks (Bar et al., 2006; Ingjaldsson et al.,
2003; Kahkonen and Bondarenko, 2000; Rechlin et al., 1996; Thayer
et al., 2006). Because studies associating ANS with alcohol use are
restricted to heavy drinkers without AD, and studies on AD only
included non-drinkers with severe AD, much is still unclear about
the unique contribution of alcohol use and AD to sympathetic and
parasympathetic control of the heart.

To our knowledge, this study is the first in examining the HPA-
axis as well as ANS in a large sample of persons with no, remitted,
and current AD who also differ in the amount of alcohol use (no,
moderate, heavy alcohol use). Consequently, we are able to assess
the relative contribution of alcohol use and AD to dysregulation of
both stress systems. In order to examine HPA-axis and ANS indica-
tors in a broad range of alcohol dependent persons, persons with a
DSM-IV diagnosis of AD are not restricted to the specific subgroup
of severely addicted inpatients as in previous studies. In addition,
the current study is sufficiently powered to examine confounding
by socio-demographics, health indicators and depressive or anxiety
disorders.

2. Methods

2.1. Study sample

Data were derived from the Netherlands Study of Depression and Anxiety
(NESDA) (Penninx et al., 2008), an ongoing longitudinal cohort study among 2981
adults (18–65 years), from which 94.8% were of North-European ancestry. Partici-
ants were recruited from the community (10%), from primary care (54%) through a
screening procedure conducted among 65 General Practitioners, and from special-
ized mental health care (27%) when newly enrolled at one of the 17 participating
sites (see Section 2.2.3), whereas participants collected their saliva samples for
home shortly after the interview (see Section 2.2.2). Participants were compensated with a small incentive (gift certificate of 15
€) and was administered by specially trained research staff. A distinction was made between no AD, remitted AD (lifetime AD but not in the last 12 months) and current AD (meeting AD criteria in the last 12 months).

2.2. Measurements

2.2.1. Alcohol variables. Alcohol use – Alcohol use was assessed by two items of the Alcohol Use Disorders Identification Test (AUDIT) questionnaire (Babor and Fuente,
1992) concerning the average frequency of drinking and the amount of drinks on a
typical drinking day in the past year. From these items, we derived the average
amount of alcoholic drinks/day, in which one drink refers to one glass of a
drink containing alcohol. We, subsequently, distinguished between non-drinkers (0
drinks/day), moderate drinkers (men: ≤3; women: ≤2 drinks/day) and heavy
drinkers (men: >3; women: >2 drinks/day), based on general guidelines that are
used in health organizations in the Netherlands (Stuurgroep Multidisciplinaire
Richtlijnontwikkeling GGZ, 2009) and in other studies on this topic (e.g., Gianoulakis
et al., 2003).

AD status – A diagnosis of alcohol dependence was established with the
Composite International Diagnostic Interview (CIDI), version 2.1 (World Health
Organization, 1997), which classifies diagnoses according to DSM-IV criteria (American Psychiatric Association, 2000). The CIDI is reliable and valid in assess-
ing AD (van den Brink, 2005; van den Brink et al., 1997) and was administered by specially trained research staff. A distinction was made between no AD, remitted AD (lifetime AD but not in the last 12 months) and current AD (meeting AD criteria in the last 12 months).

2.2.2. Hypothalamic–pituitary–adrenal (HPA)/axis. As described in more detail else-
where, questionnaires were instructed to collect saliva samples at home on (Vreeburg
et al., 2009a,b) a regular (preferably working) day, shortly after the interview. The
time interval between the interview and saliva sampling was 9.0 days (25th–75th
percentile: 4–22). Saliva samples were obtained using Salivettes (Starstedt, Ger-
many) at seven time points. The cortisol awakening response (CAR) includes four
sampling points: one at awakening (T1) and the other three 30 (T2), 45 (T3) and 60
(T4) min later. Two evening cortisol values were collected: one at 22:00 h (T5) and
one at 23:00 h (T6). Dexamethasone suppression was measured by cortisol sam-
ping the next morning at awakening (T7) after ingestion of 0.5 mg dexamethasone
the evening before. The saliva samples were stored in refrigerators and returned by mail. After receipt, Salivettes were centrifuged at 0.0 °C for 10 min,
 aliquoted and stored at −80 °C. Cortisol analysis was performed by competitive
electrochemiluminescence immunoassay (E170 Roche, Switzerland), as described
in Van Aken et al. (2003). The functional detection limit was 2.0 nmol/l and the intra-
and inter-assay variability coefficients in the measuring range were less than 10%. Data cleaning excluded values ≥2 SD above the mean (i.e. ≥59.6–123.6 nmol/l
for T1–T4, 40.9 nmol/l for T5, 59.8 nmol/l for T6 and 35.6 nmol/l for T7).

Cortisol awakening response – The area under the curve with respect to the
ground (AUCg) and the area under the curve with respect to the increase (AUCi)
were calculated using the formula described by Pruessner et al. (2003). The
AUCg is an estimate of the total cortisol secretion over the first hour after awakening,
whereas the AUCi is a measure of the dynamic of the cortisol awakening response.
Both measures require all four morning samples (n = 1723).

Evening cortisol – The mean of the two evening measures was used to reflect
evening cortisol level. At least one evening cortisol value was required (n = 2015).

Cortisol suppression test – We used a cortisol suppression ratio calculated by
cortisol awakening on the first day (T1) divided by cortisol value at awakening
the next day (T7) after ingestion of 0.5 mg dexamethasone the evening before. Only
those persons who reported that they had ingested dexamethasone were included
in these analyses (n = 1936).

2.2.3. Autonomic nervous system (ANS). During their visit to the research centers,
participants were requested to wear the Vrije Universiteit Ambulatory Monitoring
System (VU-AMS; Vrije Universiteit, Amsterdam, the Netherlands). The VU-AMS is a
light-weight portable device that records electrocardiograms (ECG) and changes in
thorax impedance (dIZ) from six surface electrodes placed at the chest and back of
the participant (De Geus et al., 1995; Willemsen et al., 1996). With this device we
could establish respiratory sinus arrhythmia (RSA), heart rate (HR), and pre-ejection
period (PEP).

RSA – RSA reflects cardiac parasympathetic control (high RSA reflecting high
parasympathetic control), and was obtained by combining the inter-beat interval
(Adam et al., 2006; Badrick et al., 2008). Although heavy alcohol
use has been associated with hyperactivity of the HPA-axis, AD has
been linked to a blunted stress response for at least four weeks post-
withdrawal (Bernardy et al., 1996; Errico et al., 1993; Lovallo et al.,
2000; Sinha et al., 2009). However, these studies in persons with AD have been restricted to those who are abstinent from alcohol and, therefore, could not clarify the relative contributing role of alcohol use and AD to activity of the HPA-axis. A further limitation of these previous studies is that only inpatients severely dependent
on alcohol were included.

With regard to the ANS analyses, 118 persons were excluded because no ANS
data were available, leaving a sample of 2863 (96.0%) persons. Persons with valid
ANS data were slightly younger (41.8 years versus 44.0 years, p = 06), but did not differ with respect to gender (p = 06), education, alcohol use (p = 13) and AD status (p = 05) from excluded persons.
time series with the filtered (0.1–0.4 Hz) dZ signal which corresponds to the respiration signal. RSA was obtained by subtracting the shortest interbeat-interval (IBI) during heart rate acceleration in the inspiratory phase from the longest IBI during deceleration in the expirational phase for all breaths (n = 2863).

**HR** – The interbeat interval time series was extracted from the ECG signal to obtain HR, as an indicator of both sympathetic and parasympathetic control over the heart (n = 2863).

**PEP** – PEP reflects cardiac sympathetic control (high PEP reflecting low sympathetic control) as the noradrenergic inotropic drive to the left ventricle and was obtained from the dZ/dt signal, ensemble averaged across one-minute periods time-locked to the R-wave of the ECG. PEP was defined as the interval from the B-point (upstroke) to the X-point (inclusura) of the dZ/dt signal (n = 2829).

### 2.2.4. Covariates

Sociodemographics, health and sampling factors as well as depression/anxiety-related characteristics were considered as covariates as they have been linked with alcohol use and/or alcohol dependence as well as with the HPA-axis and/or the ANS in previous studies. Socio-demographic factors included sex, age and years of education. Body mass index (BMI) was calculated as weight divided by length in meters squared. Physical activity was assessed with the International Physical Activity Questionnaire (Craig et al., 2003) and expressed in 1000 Metabolic Equivalent (MET)-minutes in the past week. Information about past and current smoking was obtained during the interview. Cardiovascular disease (including coronary disease, cardiac arrhythmia, angina pectoris, heart failure and myocardial infarction) and number of other chronic conditions (diabetes, osteoarthritis, stroke, cancer, chronic lung disease, thyroid disease, liver disease, chronic fatigue syndrome, intestinal disorders and ulcers) were ascertained by self-report. Furthermore, use of heart medication was assessed by drug container inspection of medication used in the past month and classified according to the World Health Organizations Anatomical Therapeutic Chemical (ATC) coding system. Both use of beta-blockers (ATC code C07, used at least 50% of the time) and use of other cardiovascular medication (ATC-codes C01 [cardiac therapy], C02 [antihypertensives], C03 [diuretics], C04 [peripheral vasodilators], C05 [vasoactive], C08 [calcium channel blockers] or C09 [agents acting on the renin-angiotensin system]) were ascertained. Diagnoses of DSM-IV remitted (lifetime but not in the last 12 months) or current (in the last 12 months) depression (major depressive disorder and dysthymia) and anxiety (generalized anxiety disorder, social phobia, panic disorder and agoraphobia) disorders were established with the Composite International Diagnostic Interview (CIDI, version 2.1 (World Health Organization, 1997). Use of selective serotonin re-uptake inhibitors (SSRIs; ATC code N06AB), tricyclic antidepressants (TCAs; ATC code N06BA) and other antidepressants (N06AF and N06AX, mainly venlafaxine and mirtazapine) were also ascertained. Additionally, for analyses of cortisol measures, sampling factors that have been shown to influence cortisol measures by Vreeburg et al. (2009b), were included. Participants reported time of awakening and working status on the sampling day. Season was categorized into dark months (October through February) and months with more daylight (March through September). Average sleep duration during the last week was dichotomized into sleeping more or less than six hours a night.

### 2.3. Statistical analysis

Analyses were conducted using SPSS version 15.0 statistical software. All HPA-axis and ANS measures showed normal distributions except for the evening cortisol level and the cortisol suppression ratio, which were log transformed before analyses and back-transformed to report in tables and figures. Baseline characteristics were compared across groups based on alcohol use (no, moderate, heavy alcohol use) and AD status (no, remitted, current AD) using t-statistics for categorical variables and analysis of variance for continuous variables. To examine whether HPA-axis measures (i.e. AUCg, AUCi, evening cortisol level and cortisol suppression ratio) and ANS measures (i.e. HR, RSA and PEP) differed across groups based on alcohol use (reference: moderate drinkers) and AD status (reference: no AD), we used analyses of (co)variance. Results of unadjusted analyses as well as adjusted analyses controlling for covariates are presented. In addition to separately relating the alcohol variables with HPA-axis and ANS measures, we also examined the relative contribution of alcohol use and AD status. Therefore, we tested whether the patterns of HPA-axis and ANS activity across the three alcohol use groups differed between persons with and without lifetime AD.

### 3. Results

#### 3.1. Sample

Mean age of the sample was 43.3 (SD = 13.0) years and 65.3% were women. The distribution of alcohol use was as follows: 15.5% were non-drinkers (mean = 0.0, SD = 0.0 drinks/day; see Table 1), 73.2% were moderate drinkers (mean = 0.8, SD = 0.7 drinks/day) and 11.3% were heavy drinkers (mean = 4.0, SD = 1.8 drinks/day). Of the sample, 85.8% had no lifetime AD (mean = 0.8, SD = 1.1 drinks/day), 7.8% had remitted AD (mean = 1.3, SD = 1.8 drinks/day) and 6.4% had current AD (mean = 2.8, SD = 2.3 drinks/day). Most of the sample characteristics differed significantly between alcohol use groups and between diagnostic (AD) groups (see Table 1), indicating the need for statistical adjustment of differences in outcomes.

#### 3.2. HPA-axis measures

Table 2 presents results from unadjusted and adjusted analyses, associating HPA-axis activity with alcohol use and AD status.

**Cortisol awakening response (CAR): AUCg** – Heavy drinkers had an increased AUCg compared to moderate drinkers in both unadjusted and adjusted analyses (adjusted: $p = .02$). No significant differences in AUCg were found between non-drinkers and moderate drinkers (adjusted: $p = .75$). Although persons with remitted and current AD had a higher AUCg compared to persons without AD in the unadjusted analyses ($p = .08$ and $p = .04$, respectively), these associations were not significant after adjustment for covariates (adjusted: $p = .70$ and $p = .68$, respectively).

**Cortisol awakening response (CAR): AUCi** – No significant associations were found between alcohol use levels or AD status and AUCi in the unadjusted or adjusted analyses.

**Evening cortisol** – In both unadjusted and adjusted analyses, heavy drinkers had an increased evening cortisol level compared to moderate drinkers (adjusted: $p = .006$), whereas non-drinkers did not differ from moderate drinkers (adjusted: $p = .29$). Evening cortisol levels were significantly higher among persons with remitted and current AD compared to persons without lifetime AD in unadjusted analyses ($p = .01$ and $p = .006$, respectively), but these differences were no longer significant after adjustment for potential confounders (adjusted: $p = .07$ and $p = .18$, respectively).

**Cortisol suppression ratio** – In the unadjusted analyses, non-drinkers and heavy drinkers had a significantly decreased cortisol suppression ratio relative to moderate drinkers (adjusted: $p = .04$ and $p = .03$, respectively), but the differences were no longer significant after adjustment for potential confounders (adjusted: $p = .07$ and $p = .18$, respectively). No significant associations were found between AD status and the cortisol suppression ratio in both unadjusted and adjusted analyses.

#### 3.3. ANS measures

Results from both unadjusted and adjusted analyses associating alcohol use and AD status with ANS measures are also presented in Table 2.

##### 3.3.1. Parasympathetic control. RSA

In the unadjusted analyses concerning alcohol use and RSA, we initially found an inverted U-curve in which non-drinkers and heavy drinkers had a significantly decreased RSA compared to moderate drinkers (both: $p < .001$). However, in the adjusted analyses this decrease only remained significant for non-drinkers (adjusted: $p = .05$) and not for heavy drinkers (adjusted: $p = .37$). No significant associations were found between AD status and RSA.

##### 3.3.2. Parasympathetic/sympathetic control. HR

Adjusted analyses concerning alcohol use showed a U-curve in which non-drinkers and heavy drinkers had a significantly increased HR compared to moderate drinkers (adjusted: $p = .006$ and $p = .04$, respectively). Persons with a remitted and current AD did not significantly differ in HR from persons without AD in both unadjusted and adjusted analyses.
analyses (see Table 2). The figures show similar patterns of the HPA-axis and ANS indicators across alcohol use groups in persons with and without lifetime AD. In addition, persons with and without a lifetime AD diagnosis. Fig. 1 shows the results of adjusted analyses relating alcohol use to those HPA-axis (i.e. AUCg and evening cortisol) and ANS measures (i.e. RSA, HR, PEP) that showed a significant \( p < 0.05 \) association with alcohol use in the previous analyses (see Table 2). The figures show similar patterns of the HPA-axis and ANS indicators across alcohol use groups in persons with and without a lifetime AD diagnosis. In addition, persons with and without lifetime AD did not significantly differ on any of the HPA-axis and ANS indicators within subgroups based on alcohol use (within non-drinkers: all \( p-values > 0.29 \); within moderate drinkers: all \( p-values > 0.41 \); within heavy drinkers: all \( p-values > 0.62 \)). In sum, these findings confirm that it is indeed alcohol use, and not lifetime AD, that is driving the association.

### 4. Discussion

The present study shows that alcohol use, rather than alcohol dependence (AD), is associated with dysregulation of the hypothalamic–pituitary–adrenal (HPA)-axis as well as the autonomic nervous system (ANS). On the one hand, we found that persons with a remitted or current AD did not differ from persons without a lifetime diagnosis of AD on any of the HPA-axis and ANS indicators. On the other hand, heavy alcohol use was associated with hyperactivity of the HPA-axis and increased sympathetic control of the heart compared to moderate alcohol use. The presence of AD did not change the association of alcohol use with both stress systems as similar patterns of HPA-axis activity and cardiac parasympathetic/sympathetic control across alcohol use groups were found in persons with and without lifetime AD.

To our knowledge, this study is the first to examine the relative contribution of alcohol use and AD to the HPA-axis and ANS. Previous studies have shown that heavy alcohol use was associated with hyperactivity of the HPA-axis (Adam et al., 2006; Badrick et al., 2008; Gianoulakis et al., 2003; Thayer et al., 2006) and increased sympathetic control of the ANS (Ohira et al., 2009; Ryan and Howes, 2002) in persons without AD. The present study corroborates these findings, but also provides important additional evidence as we found similar associations with alcohol use in persons without as well as with a lifetime diagnosis of AD. In our sample, alcohol use, and not AD, is associated with dysregulation of the HPA-axis and ANS and may account for the various physiological, psychological

### Table 1

Sample characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Alcohol use</th>
<th>Alcohol dependence (AD)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>No (n = 498)</td>
<td>Moderate (n = 2112)</td>
<td>Heavy (n = 337)</td>
<td>( p ^ a )</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>383 (76.9%)</td>
<td>1350 (63.9%)</td>
<td>226 (67.1%)</td>
</tr>
<tr>
<td>Age in years, mean (SD)</td>
<td>42.7 (12.8)</td>
<td>40.5 (13.1)</td>
<td>46.6 (12.3)</td>
</tr>
<tr>
<td>Education in years, mean (SD)</td>
<td>11.0 (3.2)</td>
<td>12.4 (3.2)</td>
<td>12.6 (3.5)</td>
</tr>
<tr>
<td>Health and sampling factors</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index, mean (SD)</td>
<td>26.7 (5.8)</td>
<td>25.4 (4.9)</td>
<td>25.3 (4.5)</td>
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<tr>
<td>Physical activity/1000 MET-min/week, mean (SD)</td>
<td>3.6 (3.2)</td>
<td>3.7 (3.0)</td>
<td>3.4 (3.0)</td>
</tr>
<tr>
<td>Smoking</td>
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</tr>
<tr>
<td>No, n (%)</td>
<td>198 (39.8%)</td>
<td>585 (27.7%)</td>
<td>45 (13.4%)</td>
</tr>
<tr>
<td>Past, n (%)</td>
<td>138 (27.7%)</td>
<td>727 (34.4%)</td>
<td>120 (35.6%)</td>
</tr>
<tr>
<td>Current, n (%)</td>
<td>162 (32.5%)</td>
<td>800 (37.9%)</td>
<td>172 (51.0%)</td>
</tr>
<tr>
<td>Cardiovascular disease, n (%)</td>
<td>33 (6.6%)</td>
<td>122 (5.8%)</td>
<td>21 (6.2%)</td>
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<tr>
<td>Number of other chronic diseases, mean (SD)</td>
<td>1.3 (1.2)</td>
<td>0.8 (1.0)</td>
<td>0.9 (1.2)</td>
</tr>
<tr>
<td>Use of beta-blockers, n (%)</td>
<td>52 (10.4%)</td>
<td>144 (6.8%)</td>
<td>33 (9.8%)</td>
</tr>
<tr>
<td>Use of other heart medication, n (%)</td>
<td>62 (12.4%)</td>
<td>219 (10.4%)</td>
<td>37 (11.0%)</td>
</tr>
<tr>
<td>Time of awakening, mean (SD)</td>
<td>7h23 (1h01)</td>
<td>7h27 (1h02)</td>
<td>7h37 (1h11)</td>
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<td>Working on sampling day, n (%)</td>
<td>321 (64.5%)</td>
<td>1550 (73.4%)</td>
<td>228 (67.7%)</td>
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<tr>
<td>Month with more daylight, n (%)</td>
<td>350 (70.3%)</td>
<td>1442 (68.3%)</td>
<td>225 (66.8%)</td>
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<tr>
<td>Sleep (≤ 6h of sleep), n (%)</td>
<td>142 (28.5%)</td>
<td>493 (23.3%)</td>
<td>95 (28.2%)</td>
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<td>Depression/anxiety-related characteristics</td>
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<td>Depressive disorder</td>
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<td>131 (26.3%)</td>
<td>750 (35.5%)</td>
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<td>105 (21.1%)</td>
<td>511 (24.2%)</td>
<td>76 (22.6%)</td>
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<td>Current, n (%)</td>
<td>262 (52.6%)</td>
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<td>907 (42.9%)</td>
<td>136 (40.4%)</td>
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<td>Current, n (%)</td>
<td>286 (57.4%)</td>
<td>907 (42.9%)</td>
<td>156 (46.3%)</td>
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<td>Use of SSRI, n (%)</td>
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<td>308 (14.6%)</td>
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<td>40 (1.9%)</td>
<td>9 (2.7%)</td>
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<td>110 (5.2%)</td>
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<td>Alcohol use</td>
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<td>4.0 (1.8)</td>
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</tbody>
</table>

Abbreviations: SSRI, selective serotonin re-uptake inhibitors; TCA, tricyclic antidepressants.

\( p ^ a \) -Value based on \( \chi ^ 2 \)-statistics (dichotomous and categorical variables) or analysis of variance (continuous variables).
and behavioral problems – such as cardiovascular disease, depressive/anxiety disorders and aggressive behavior – that are related to alcohol.

As findings of previous studies suggested that persons with AD have impaired inhibitory control, resulting in a blunted cortisol response and lower parasympathetic control of the ANS (Bar et al., 2006; Bernardy et al., 1996; Errico et al., 1993; Ingjalddson et al., 2003; Kahlkon and Bondarenko, 2000; Lovaletter et al., 2000; Rechlin et al., 1996; Thayer et al., 2006), whereas we did not find such an association, this may imply that the HPA-axis and ANS are only dysregulated in specific subgroups of alcohol dependent persons. Possibly, the extent of dysregulation may depend on the severity of the underlying addictive process, as was also suggested by others (Junghanns et al., 2003; Sinha et al., 2009). Therefore, our study provides important, additional information as we included a sample of regular alcohol dependent persons who have not been addressed in previous studies among severely dependent inpatients. We, therefore, conclude that there are no dysregulations of stress systems in non-treatment seeking alcohol dependent persons, although we cannot rule out that severely alcohol dependent inpatients may differ from our sample in this respect.

The current study has both strengths and limitations. The main strengths are the relatively large sample size and the presence of persons with and without AD, who also differed in their level of alcohol use. In addition, we examined the HPA-axis as well as ANS, which is unique in studies on alcohol use and AD. In contrast with previous studies, our study was also sufficiently powered to examine confounding by socio-demographics, health indicators and depression/anxiety-related characteristics. However, an important limitation of our study is that alcohol use and the presence of AD were based on self-report only. In addition, alcohol use and current AD were based on a longer time frame (last 12 months) than in other studies, limiting conclusions with regard to the temporal relationship between stress indicators and the level of alcohol use or status of AD to the very moment they were assessed. With regard to the cortisol suppression ratio, it is impossible to guarantee ingestion of the dexamethasone pill by all persons. Therefore, within a small validation study we had measured dexamethasone levels with a radioimmunoassay using the anti-dexamethasone antibody from IgG Corporation (Nashville, TN, functional detection limit is 0.4 nmol/l and reported cross-reactivity for cortisol is 0.04%).

Abbreviations: CAR, cortisol awakening response; AUCg/i, area under the curve with respect to the ground/increase; RSA, respiratory sinus arrhythmia; HR, heart rate; PEP, pre-ejection period.

### Table 2

Results of Analyses of Covariance associating alcohol use and alcohol dependence with HPA-axis and ANS activity.

<table>
<thead>
<tr>
<th>Alcohol use</th>
<th>No (Mean (SE))</th>
<th>p&lt;sub&gt;a&lt;/sub&gt;</th>
<th>Moderate (Mean (SE))</th>
<th>p&lt;sub&gt;b&lt;/sub&gt;</th>
<th>Heavy (Mean (SE))</th>
<th>p&lt;sub&gt;c&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPA-axis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAR: AUCg, nmol/l/h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>18.6 (0.4)</td>
<td>.47</td>
<td>18.9 (0.2)</td>
<td>&lt;.001</td>
<td>20.6 (0.5)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Adjusted</td>
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<td>.75</td>
<td>19.0 (0.2)</td>
<td>.02</td>
<td>20.3 (0.5)</td>
<td>.03</td>
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<tr>
<td>CAR: AUCi, nmol/l/h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>2.6 (0.4)</td>
<td>.28</td>
<td>2.1 (0.2)</td>
<td>.07</td>
<td>3.0 (0.5)</td>
<td>.07</td>
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<td>2.1 (0.2)</td>
<td>.11</td>
<td>2.9 (0.5)</td>
<td>.11</td>
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<tr>
<td>Evening cortisol, nmol/l/h</td>
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<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>4.6 (1.0)</td>
<td>.68</td>
<td>4.6 (1.0)</td>
<td>&lt;.001</td>
<td>5.5 (1.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Adjusted</td>
<td>4.8 (1.0)</td>
<td>.29</td>
<td>4.6 (1.0)</td>
<td>.06</td>
<td>5.0 (1.0)</td>
<td>.06</td>
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<tr>
<td>Cortisol suppression ratio</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>2.3 (1.0)</td>
<td>.04</td>
<td>2.4 (1.0)</td>
<td>.03</td>
<td>2.3 (1.0)</td>
<td>.18</td>
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<tr>
<td>Adjusted</td>
<td>2.3 (1.0)</td>
<td>.07</td>
<td>2.4 (1.0)</td>
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<td>ANS</td>
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<td></td>
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<td>Parasympathetic control</td>
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<td></td>
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<tr>
<td>RSA, ms</td>
<td>40.6 (1.2)</td>
<td>&lt;.001</td>
<td>46.1 (0.6)</td>
<td>&lt;.001</td>
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<td>&lt;.001</td>
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<tr>
<td>Adjusted</td>
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<td>.05</td>
<td>44.9 (0.5)</td>
<td>.37</td>
<td>43.8 (1.2)</td>
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<tr>
<td>HR, bpm</td>
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<td>&lt;.001</td>
<td>71.6 (0.2)</td>
<td>.56</td>
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<td>.56</td>
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<td>71.6 (0.2)</td>
<td>.04</td>
<td>72.7 (0.5)</td>
<td>.04</td>
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<tr>
<td>Sympathetic control</td>
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<tr>
<td>PEP, ms</td>
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<td>.16</td>
<td>119.6 (0.4)</td>
<td>.34</td>
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<tr>
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<td>119.6 (0.4)</td>
<td>.04</td>
<td>117.5 (1.0)</td>
<td>.04</td>
</tr>
</tbody>
</table>

### Alcohol dependence (AD)

<table>
<thead>
<tr>
<th>Alcohol use</th>
<th>No (Mean (SE))</th>
<th>p&lt;sub&gt;d&lt;/sub&gt;</th>
<th>Remitted (Mean (SE))</th>
<th>p&lt;sub&gt;e&lt;/sub&gt;</th>
<th>Current (Mean (SE))</th>
<th>p&lt;sub&gt;f&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPA-axis</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>CAR: AUCg, nmol/l/h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>18.9 (0.2)</td>
<td>.08</td>
<td>20.0 (0.6)</td>
<td>.08</td>
<td>20.4 (0.7)</td>
<td>.04</td>
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<tr>
<td>Adjusted</td>
<td>19.0 (0.2)</td>
<td>.70</td>
<td>19.3 (0.6)</td>
<td>.70</td>
<td>19.3 (0.7)</td>
<td>.68</td>
</tr>
<tr>
<td>CAR: AUCi, nmol/l/h</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>2.2 (0.2)</td>
<td>.94</td>
<td>2.3 (0.5)</td>
<td>.94</td>
<td>2.6 (0.6)</td>
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<tr>
<td>Adjusted</td>
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<td>.79</td>
<td>2.1 (0.5)</td>
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<tr>
<td>Evening cortisol, nmol/l/h</td>
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<tr>
<td>Unadjusted</td>
<td>4.8 (1.0)</td>
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<td>5.2 (1.0)</td>
<td>.01</td>
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<td>.006</td>
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<tr>
<td>Adjusted</td>
<td>4.7 (1.0)</td>
<td>.67</td>
<td>4.7 (1.0)</td>
<td>.67</td>
<td>4.8 (1.0)</td>
<td>.65</td>
</tr>
<tr>
<td>Cortisol suppression ratio</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>2.4 (1.0)</td>
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<td>2.4 (1.0)</td>
<td>.33</td>
<td>2.4 (1.0)</td>
<td>.61</td>
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<tr>
<td>Adjusted</td>
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<td>.86</td>
<td>2.4 (1.0)</td>
<td>.86</td>
<td>2.4 (1.0)</td>
<td>.76</td>
</tr>
</tbody>
</table>
could, therefore, determine the relative contribution of both alcohol use and AD on the HPA-axis and ANS. Heavy alcohol use, and not AD, was associated with dysregulations of both stress systems resulting in hyperactivity of the HPA-axis and increased sympathetic control of the heart. Consequently, dysregulations in both stress systems may explain the harmful physiological, psychological and behavioral consequences as seen in heavy drinkers.

Conflicts of interest

All authors declare that they have no conflicts of interest.

Role of funding source

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Contributors

All authors have contributed to the design of the study. Carmilla M.M. Licht and Sophie A. Vreeburg prepared the data of the ANS and HPA-axis measures. Lynn Boschloo managed the literature searches, undertook the statistical analysis and wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.