Determinants and consequences of long-term benzodiazepine use

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Long-term Benzodiazepine Use and salivary Cortisol: the Netherlands Study of Depression and Anxiety (NESDA)

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Journal of Clinical Psychopharmacology
2010, 30 (2): 160-168

ABSTRACT

Background: As benzodiazepines (BZDs) have anxiolytic effects, it is expected that they influence the stress system. During short-term treatment, BZD use was found to suppress cortisol levels. However, little research has been done on the effects of long-term BZD administration on the hypothalamic-pituitary-adrenal (HPA) axis.

Methods: The association between long-term BZD use and cortisol levels was investigated in subjects of the Netherlands Study of Depression and Anxiety with a lifetime diagnosis of anxiety or depression (n=1531). Subjects were categorized as "daily BZD users" (n=96), "infrequent BZD users" (n=172) and "nonusers" (n=1263). Possible associations between characteristics of BZD use (dose, duration, and dependence) and salivary cortisol levels were analyzed.

Main outcome measure: Subjects provided 7 saliva samples, from which 4 cortisol indicators were calculated: the cortisol awakening response, diurnal slope, evening cortisol, and cortisol suppression after ingestion of 0.5 mg dexamethasone.

Results: Daily users used BZDs for a median duration of 26.5 months and had a median daily dosage of 6.0 mg as measured in diazepam equivalents. Evening cortisol levels were significantly lower in daily users (P=0.004, effect size: d=0.24) and infrequent users (P=0.04, effect size: d=0.12) as compared to nonusers. We did not find significant differences in the cortisol awakening response, diurnal slope or in the dexamethasone suppression test.

Conclusions: Despite the finding of slightly lower evening cortisol levels in daily and infrequent BZD users as compared to nonusers, results indicate that long-term BZD use is not convincingly associated with HPA axis alterations.

INTRODUCTION

As benzodiazepines (BZDs) have anxiolytic and sedating effects, it is expected that they influence the stress system. Most studies on the effects of short-term BZD treatment (maximum of 3 months) on the hypothalamic-pituitary-adrenal (HPA) axis in human subjects reported a decrease in cortisol levels,[1-11] although some studies reported mixed results.[12,13] These inconsistencies may be explained by differences in dosages and half-lives of the BZDs used[13] and by disparities in the measurement time points used in the assessments (only predrug and postdrug measurements,[13] at certain time intervals,[6,8,10-12,14] or for a full circadian cycle[1,2,5]). Differences in patient groups,[12,13] and measurements of basal versus stress-provoked cortisol levels may also influence the results.[3,13] In general, the studies measured plasma cortisol levels[1-3,5,6,9,11,13] or urinary free cortisol as measures of HPA axis activity.[4] Associations between BZD use and dexamethasone suppression have only been investigated in 1 study and no clear effect of BZD use on dexamethasone suppression was observed.[14] A few studies found that the cortisol decrease in response to BZD treatment was followed by a return to baseline cortisol levels within only a few hours, despite persisting high plasma drug levels,[15-17] suggesting fast development of tolerance to the stress-axis-suppressing effects of BZDs. In contrast, other studies did report significant cortisol reductions in 24h, overnight and daytime means,[1] suggesting that tolerance does not develop as rapidly.

Tolerance to the effects of BZDs as a consequence of chronic use (>3 months) has been extensively discussed in previous studies.[18,19] In related research on the therapeutic effects of BZDs, several authors reported that tolerance was developed to only the cognitive and psychomotor effects and not to the anxiolytic effects of chronic BZD treatment,[19] whereas others found decreasing anxiolytic efficacy as well when treatment exceeded a few weeks.[18] Most studies on the effects of

BZDs on cortisol levels found that cortisol suppression was maintained for up to 3 months of use.[1,2,4,9,12]

There was only 1 small cross-sectional study investigating long-term BZD use (> 3 months).[20] The authors found that long-term users have similar baseline cortisol levels as nonusers, indicating that BZDs do not maintain their cortisol-suppressing effects during longer-term use. In contrast, an additional dosage of BZDs (on top of the BZD dosage that chronic users took daily) still affected the HPA axis after chronic use. However, comparison groups were small, no measurement of the whole circadian rhythm was conducted, and no dexamethasone challenge test was applied.[20]

In this paper, we examine the effects of chronic BZD use on various salivary cortisol measures (cortisol awakening response, diurnal slope, evening cortisol level and suppression after oral dexamethasone administration). In addition, we explore the effects of dosage, duration of use, and level of dependence. The study was carried out on data from 1531 subjects with a lifetime diagnosis of anxiety and / or depression participating in the Netherlands Study of Depression and Anxiety (NESDA).

MATERIALS AND METHODS

Subjects

Subjects participated in the baseline assessment of NESDA, an 8-year longitudinal cohort study of 2981 respondents aged 18 to 65 years.[21] Subjects were recruited from the community, general practice and specialized mental health care institutions throughout the Netherlands. Subjects completed a medical exam, an in-person interview, saliva collection and several questionnaires. The study protocol was approved by the Ethical Review Board of each participating center and all subjects signed an informed consent at the baseline assessment.

To investigate the associations between BZD use and salivary cortisol indicators, 3 groups were defined: subjects who reported daily BZD use in the month prior to the baseline interview ("daily BZD users", n=176), subjects who used BZDs on an infrequent basis in the previous month ("infrequent BZD users", n=264) and those reporting no use of BZDs in the last month ("nonusers", n=1854). All subjects reported a current or past diagnosis of a depressive or anxiety disorder (referred to as a lifetime disorder), defined as an anxiety disorder (panic disorder with or without agoraphobia, generalized anxiety disorder or social phobia) or depressive disorder (dysthymia or Major Depressive Disorder, MDD) as assessed by the Composite International Diagnostic Interview (WHO version 2.1) which classifies diagnoses according to the criteria of the Diagnostic and Statistic Manual of Mental Disorders IV-TR (American Psychiatric Association, 2001). From these 3 groups, 1664 (72.5%) subjects returned saliva samples. Responders on saliva collection did not differ from non-responders in gender (67.7% vs 68.3% women, P = 0.79) but were older (43.6 ± 12.5 years vs 37.9 ± 11.9 years, P<0.001), more educated (12.2 ± 3.3 years vs 11.5 ± 3.2 years, P<0.001) and less likely to have a lifetime diagnosis of comorbid disorder (55.5% vs 64.0%, P<0.001). Furthermore, responders had marginally significantly lower rates of BZD use (18.2% vs 21.7%, P=0.06). Of the responders, 1658 provided sufficient cortisol samples of high quality from which at least one usable salivary cortisol indicator (cortisol awakening response [CAR], diurnal slope, evening cortisol or dexamethasone suppression test [DST], see later section) could be calculated.

Because of known associations with cortisol or use of BZDs, pregnant or breastfeeding women (n=10), subjects using corticosteroids (n=104), and patients with epilepsy (n=13) were excluded, leaving a final sample of 1531 subjects (1263 nonusers, 172 infrequent BZD users and 96 daily BZD users).

MEASURES

Benzodiazepine Use

Four indicators of BZD use were investigated: type of BZD, daily BZD dose, duration of BZD use, and BZD dependence severity. BZD use during the month before the baseline interview was registered by observation of drug containers brought to the interview (73.4%) or self-report (26.6%). Daily and infrequent BZD users reported the type and dosage of BZD taken on an average day of use. Frequency of use for infrequent users was taken into account when calculating the average daily dose. The daily BZD dose was computed according to the coding system of the Anatomical Therapeutic Code (ATC) and Defined Daily Dose (DDD) system.[22] The mean daily dose was calculated by dividing individual daily doses (in milligrams) of BZDs by the DDD for the particular BZD. BZDs were classified as ATC-coded groups N05BA, N05CD, and N03AE01. The non-BZD hypnotics zopiclone and zolpidem (ATC code N05CF), were also included. Similar to BZDs, these hypnotics act on the central omega I gamma aminobutyric acid receptor. For patients using BZDs other than diazepam, an equivalent daily dose was calculated with conversion tables,[23,24] and 10 mg of diazepam were regarded equivalent to 1 mg alprazolam, 10 mg bromazepam, 0.25 mg brotizolam, 20mg clobazam, 20 mg chlordiazepoxide, 13.3 mg clorazepate, 8 mg clonazepam, 30 mg flurazepam, 1 mg loprazolam, 2 mg lorazepam, 1 mg lormetazepam, 7.5 mg midazolam, 10 mg nitrazepam, 33 mg oxazepam, 20 mg prazepam, 20 mg temazepam, 20 mg zolpidem and 13 mg zopiclone. Dosages were summed when more than 1 BZD was used. The duration of BZD use was reported in months. BZD users completed the Benzodiazepine Dependence Self-Report Questionnaire (Bendep-SRQ), a 15-item self-report questionnaire, as a measure of dependence severity. Each item was rated on a 5- point scale. Three dependence dimensions were derived: 1) awareness of problematic use, 2) preoccupation with the availability of BZDs, and 3) lack of compliance with the therapeutic regimen.[25] The Bendep-SRQ has

good scalability, reliability and validity in general practice patients,[26] and psychiatric outpatients.[27]

Salivary Cortisol

The respondents were asked to collect saliva samples at home on a regular, preferably working day, shortly after the baseline interview by using Salivettes (Sarstedt AG und Co, Nürmbrecht, Germany).[28] The median time between the interview and saliva sampling was 9 days (25th – 75th percentile: 4-22). Eating, smoking, drinking tea or coffee, or brushing teeth was prohibited within 15 minutes of sampling. Saliva was measured at seven time points (Ts): upon awakening (T1), 30 minutes (T2), 45 minutes (T3) and 60 minutes (T4) after awakening and in the evening at 10_{PM} (T5) and 11_{PM} (T6). Immediately after saliva sampling at T6, the cortisol suppression test was carried out by oral administration of a 0.5-mg dexamethasone pill and assessed by cortisol sampling the next morning directly after awakening (T7). All samples were refrigerated and returned by mail. During laboratory analysis, Salivettes were centrifuged at 2000g for 10 minutes, aliquoted, and stored at -80°C. Competitive electrochemiluminescence immunoassay (E170 Roche, Basel, Switzerland) was used to measure cortisol levels at a functional detection limit of 2.0 nmol/l.[29] Intraassay and interassay variability coefficients in the measuring range were less than 10%. Assays were repeated if cortisol levels were very high (> 80 nmol/L) or very low (< 1 nmol/L) (n=128). All very high samples remained high in the second measurement, and the mean of the 2 measured values was used in further analyses. In 80% of the very low samples, the repeated cortisol value was within the reference range and was used for analysis. In cases where the second measurement was also very low, the mean of the samples was used. Data cleaning was performed by excluding cortisol values more than 2 SDs above the mean.[28]

Four cortisol measures were derived: the CAR, diurnal slope, evening cortisol and cortisol suppression on the DST.[28]

Cortisol Awakening Response (CAR)

The CAR was calculated from 4 sampling points: T1, T2, T3, and T4. In our study, it was calculated by analysis of T1 to T4 with Linear Mixed Models (LMM) and 2 aggregate indicators: area under the curve with respect to the ground (AUCg) and with respect to the increase (AUCi) according to Pruessner's formulas.[30] The AUCg is an estimate of the total cortisol secretion and predicts mean cortisol levels throughout the day, and the AUCi is a measure of the dynamics of the CAR, related to the sensitivity of the system and emphasizing changes over time.[28,30] For the AUC analyses, a minimum of 3 samples were required. For those with 1 missing cortisol value (n=84), the fourth was imputed using linear regression analyses with information on the other available 3 cortisol values, gender, age, awakening time and smoking status.

Diurnal Slope and Evening Cortisol

As cortisol levels at 10_{PM} (T5) and 11_{PM} (T6) were correlated ($r=0.73$, $P < 0.01$), evening cortisol was defined as the average of the 2 values (T5 and T6) or by one of the 2 if only one was available. Diurnal slope was calculated by subtracting the evening cortisol level (as calculated earlier) from the cortisol level at T1 and dividing it by the time in hours between the 2 samples, resulting in the change over time of cortisol throughout the day, calculated per hour.[28,31]

Dexamethasone Suppression Test (DST)

In addition to the cortisol level at awakening after dexamethasone ingestion (T7), a cortisol suppression ratio was calculated by dividing the cortisol value at awakening on day 1 (T1) by the post-dexamethasone cortisol value at awakening on day 2 (T7). Lower post-dexamethasone cortisol levels (T7) and higher DST ratios (ie, a larger difference between T1 and T7) indicate a greater cortisol-suppressing effect of dexamethasone.

Covariates

As associations between sociodemographics (gender, age, education, and North-European ancestry), sampling factors (awakening time, work status, weekday, season, and sleep duration) and health indicators (smoking, physical activity) on salivary cortisol variables have been described previously,[32] these identified determinants were considered as covariates.

Comorbidity of anxiety and depression as well as antidepressant use have been found to be associated with salivary cortisol levels in previous research in this study sample,[28] and numbers of antidepressant use and comorbidity differed between BZD groups (Table 1). Therefore, comorbidity and antidepressant use were also included as covariates. Depression and anxiety disorders were established with the Composite International Diagnostic Interview (WHO version 2.1) which classifies diagnoses according to the criteria of the *Diagnostic and Statistic Manual of Mental Disorders Fourth Edition-Text Revision* (American Psychiatric Association, 2001). The use of antidepressants in the past month was determined by observation of drug containers brought to the baseline interview. Antidepressants were subdivided into selective serotonin re-uptake inhibitors (SSRI, ATC code N06AB), tricyclic antidepressants (TCA, ATC code N06AA), and other antidepressants (monoamine oxidase inhibitors N06AG, non-selective N06AF, and antidepressants classified as N06AX).

Respondents were asked to report time of awakening, and working status on the sampling day. Sampling date information was used to categorize weekday versus weekend day and season categorized in less daylight (October through February) and more daylight (March through September) months. Average sleep duration during the last week was dichotomized as ≤6 or >6 hours/night, and smoking status as current versus non-smoker. Physical activity was assessed using the International Physical Activity Questionnaire and expressed as activity

per 1000 MET-minutes (metabolic equivalent of number of calories spent by a person per minute) a week.[28]

Statistical Analyses

Characteristics of study groups were expressed by frequencies, means or medians, and compared using $\chi 2$ statistics (categorical variables), analysis of variance (continuous variables, normally distributed), and the Kruskal-Wallis-test (continuous variables, non-normally distributed). Area under the curve with respect to the increase and diurnal slope were normally distributed, which allowed data analysis with nontransformed values. T1-T4, AUCg, evening cortisol, T7, and DST were naturally log transformed because of their positively skewed distributions. Back-transformed values are given in Table 2.

Differences in AUCg, AUCi, diurnal slope, evening cortisol, T7, and DST across groups were analyzed using analysis of covariance (ANCOVA), adjusting for basic sociodemographic variables, sampling factors, health indicators, comorbidity, and antidepressant use. Cohen's *d* (the difference in group means, divided by their pooled SD) was calculated as a measure of effect size. Further analysis of the CAR was carried out with random coefficient analysis of the 4 morning cortisol data points by using LMM. This analysis keeps original values on all 4 data points, accommodates for missing data, and takes correlations between repeated measurements within subjects into account.[33]

Linear regression analyses were used to assess associations between characteristics of BZD use (ie, duration, dose and dependence as separate independent variables) and salivary cortisol indicators as continuous dependent variables after full adjustment in daily and infrequent BZD users.

Differences across the 4 most commonly used BZD types, that is, oxazepam (n=115), diazepam (n=33), alprazolam (n=16), and temazepam (n=45) on salivary cortisol indicators were analyzed in pairwise comparisons using ANCOVA, adjusting for aforementioned

covariates. The other BZDs were not included in these analyses as group numbers were to small (n<15). Oxazepam was used as reference group. Statistical significance was inferred at P < 0.05. All statistical analyses were conducted using SPSS for Windows, version 16.0 (SPSS, Chicago, Ill).

RESULTS

Characteristics of the 3 BZD user groups are presented in Table 1. BZD users were older, less educated, more often diagnosed with a comorbid disorder, and more likely to use antidepressants as compared to nonusers. Only 17.9% of subjects were short-term users (≤3 months), and the remaining 82.1% were long-term users (> 3 months). The median duration of use was 35.5 months (25th – 75th percentile: 5-96). Although the group of short-term users was too small (n = 48) to be analyzed separately, exclusion of these subjects did not affect our main results (data not shown). The median daily dosage of BZDs used was 1.0 mg (25th – 75th percentile: 0.2 – 2.0) of diazepam equivalents for infrequent users and 6.0 mg (25th – 75th percentile: 3.2 – 13.9) of diazepam equivalents for daily users. Crude saliva levels (T1-T4 and T7) did not differ between groups (Table 2).

Cortisol Awakening Response

Overall, 71.5 % of respondents showed an increase in cortisol in the first hour after awakening, with a mean increase of 6.6 nmol/L (or 53.5%). No significant effects were found for any of the crude CAR analyses (Table 2). Adjusted CAR results showed that daily users and infrequent users did not differ on overall cortisol levels from nonusers, reflected by analysis of AUCg (P=0.09 for daily users vs nonusers and P=0.74 or infrequent users vs nonusers; Table 2) and LMM analysis (daily users vs non-users, $F_{(1329, .097)}$=3,07, P=0.08; and infrequent users vs nonusers, $F_{(1413, 642)}$=0.11, P=0.74). A nonsignificant effect on AUCi (daily users vs nonusers, P=0.99

and infrequent users vs nonusers, *P*=0.99, Table 2) and no significant group by time interaction in the LMM analysis (daily users vs nonusers, $F_{(3947, 327)}$=0.49, *P*=0.69 and infrequent users vs nonusers, $F_{(4171, 422)}$=0.92, *P*=0.43) were found, indicating a similar time course between groups.

Diurnal Slope

No significant effects were found for crude or adjusted diurnal slope analyses (daily users vs non users: *P*=0.79).

Evening Cortisol Level

Unadjusted evening cortisol levels did not differ between groups (Table 2). After adjustment, evening cortisol was significantly lower in daily BZD users (*P*=0.004, effect size [Cohen's *d*], 0.24) and infrequent users (*P*=0.04, effect size, 0.12) compared to nonusers. Age and SSRI use were the most important confounders in the fully adjusted model.

Dexamethasone Suppression Test

The unadjusted cortisol suppression ratio was significantly lower in daily users as compared to nonusers (*P*=0.049, effect size, 0.08, Table 2) which indicates increased nonsuppression after dexamethasone ingestion in the daily user group. After adjustment, however, cortisol suppression ratios (*P*=0.71) and T7 levels (*P*=0.46) did not differ between groups. Infrequent users also did not differ from nonusers on either of the cortisol indicators (*P*=0.46 for cortisol suppression ratio and *P*=0.31 for T7).

Characteristics of BZD Use

Table 3 reports the results of additional analyses on specific associations between salivary cortisol levels and characteristics of BZD use (duration, dose and severity of BZD dependence as measured by the Bendep-SRQ) among the combined BZD user groups (infrequent and daily). For the duration of use, no effect on any cortisol indicator was found except

for a weak negative association with adjusted T7 cortisol levels after dexamethasone ingestion (β=-0.15, *P*=0.03), indicating that a longer duration of BZD use was associated with a somewhat lower cortisol level after dexamethasone ingestion, that is stronger suppression. The daily BZD dose and the 3 subscales of the Bendep-SRQ (problematic use, preoccupation and lack of compliance) were not associated with any salivary cortisol indicator.

Pairwise comparisons of the most common BZD types showed that the temazepam group did not differ from the oxazepam group on any of the cortisol indicators. However, the diazepam group had lower diurnal slope levels (*P*=0.01) and a decreased dexamethasone suppression ratio (*P*=0.01) as compared to oxazepam users. The alprazolam group had a lower AUCg than the oxazepam group (*P*=0.007, data not shown).

TABLE 1. Characteristics of Study Groups

	N	Nonusers n=1263	Infrequent Users n=172	Daily Users n=96	*P*
Sociodemographics					
Gender, % female	1531	67.2	71.5	58.3	0.09
Age, years	1531	42.5 (41.9-43.2)	46.0 (44.2-47.9)	48.6 (46.2-51.1)	<0.001
Education, years	1531	12.3 (12.1-12.5)	12.0 (11.5-12.5)	11.3 (10.6-11.9)	0.009
North European ancestry, %	1531	95.1	93.0	96.9	0.34
Sampling Characteristics					
Time of awakening	1531	7.27h (7.23-7.31h)	7.40h(7.28-7.52h)	7.39h(7.24-7.53h)	0.05
Working on day of sampling, %	1531	63.2	50.6	33.3	<0.001
Sampling on a weekday, %	1531	92.8	86.0	86.5	0.002
Sampling in month with more daylight hours, %	1531	56.4	64.0	47.9	0.03
≤6 hours of sleep, %	1531	27.6	41.3	41.7	<0.001
Health Indicators					
Smoking , %	1531	36.1	35.5	40.6	0.65
Physical activity (1000 MET-min/week)	1531	3.7 (3.5-3.9)	3.5 (3.1-4.0)	3.1 (2.5-3.7)	0.13
Psychiatric indicators					
Lifetime Diagnosis, %					
MDD only	1531	31.0	23.8	24.0	0.07
Anxiety only	1531	15.4	12.2	9.4	0.18
Comorbid disorder	1531	53.7	64.0	66.7	0.003
Benzodiazepine (BZD) use					
Duration of BZD use, months	268	N/A	36.0 (5.0-99.0)	26.5 (5.3-96.0)	0.29
Daily dosage of diazepam equivalents, mg*	268	N/A	1.0 (0.2-2.0)	6.0 (3.2-13.9)	<0.001
Type of BZD, %					
Oxazepam	1531	N/A	48.8	36.5	<0.001
Temazepam	1531	N/A	24.4	14.6	<0.001
Diazepam	1531	N/A	13.4	10.4	<0.001
Alprazolam	1531	N/A	2.3	14.6	<0.001
Others	1531	N/A	19.2	45.8	<0.001

Bendep SRQ					
Highly problematic use	232	N/A	8.0 (6.0-11.0)	10.0 (8.0-12.0)	<0.001
High preoccupation	232	N/A	12.0 (9.0-14.0)	15.0 (13.0-17.0)	<0.001
High lack of compliance	232	N/A	6.0 (5.0-8.0)	8.0(6.0-10.0)	<0.001
Antidepressant Use, %					
SSRI	1525	17.6	29.1	44.8	<0.001
TCA	1530	2.7	5.2	7.3	0.02
Others	1528	6.0	10.5	16.7	<0.001

Means (95% confidence intervals[CI]) are given for age, education, time of awakening, physical activity. Median (interquartile range) is given for duration of BZD use, daily dosage of BZD use, and BENDEP-SRQ as these values are not normally distributed. Percentages are given for categorical variables. *P* is derived by analysis of variance (ANOVA) for quantitative, normally distributed variables, Kruskal-Wallis test for continuous, non-normally distributed variables, or χ^2 statistics for categorical variables. Significance is inferred at $P < 0.05$. ˚Users reported the dosage of BZPs taken on an average day of use. Frequency of use has been taken into account for infrequent users.

BENDEP-SRQ indicates Benzodiazepine Dependence Self Report Questionnaire; MDD, Major Depressive Disorder; SSRI, Selective Serotonin Reuptake Inhibitor; T, Timepoint; TCA, Tricyclic Antidepressant; N/A, not applicable.

TABLE 2. Associations Between Benzodiazepine Use and Various Salivary Cortisol Indicators

Cortisol Characteristics	N	Nonusers n=1263	Infrequent Users n=172	Daily Users n=96	Nonusers vs Daily Users	Nonusers vs Infrequent Users
		Mean	Mean	Mean	P	P
Unadjusted Cortisol Characteristics		(95% CI)	(95% CI)	(95% CI)		
Cortisol Awakening Response						
Cortisol T1, at awakening, nmol/L	1517	15.6 (15.3 - 16.0)	15.8 (14.8 – 16.8)	15.2 (14.0 – 16.5)	0.53	0.77
Cortisol T2, + 30 min, nmol/L	1509	19.3 (18.8 – 19.8)	19.7 (18.4 – 21.1)	18.3 (16.8 – 20.1)	0.29	0.54
Cortisol T3, + 45 min, nmol/L	1508	17.9 (17.4 – 18.4)	18.3 (17.0 – 19.7)	16.5 (14.9 – 18.2)	0.12	0.57
Cortisol T4, + 60 min, nmol/L	1511	15.7 (15.3 – 16.2)	15.3 (14.2 – 16.5)	14.9 (13.5 – 16.4)	0.29	0.48
AUCg, nmol/L per hour	1490	18.1 (17.7 - 18.4)	18.3 (17.2 - 19.3)	17.2 (16.0 - 18.6)	0.25	0.73
AUCi, nmol/L per hour	1490	2.5 (2.1 – 2.9)	2.3 (1.3 – 3.3)	2.3 (1.0 – 3.6)	0.75	0.73
Evening Cortisol, nmol/L	1525	4.8 (4.7 – 5.0)	4.6 (4.2 – 5.0)	4.6 (4.2 – 5.2)	0.50	0.26
Dexamethasone suppression test						
Cortisol suppression ratio[1]	1452	2.4 (2.3 – 2.5)	2.4 (2.2 – 2.6)	2.2 (1.9 – 2.4)	0.049	0.91
Cortisol T7, after dexamethasone, nmol/L	1476	6.6 (6.4 – 6.8)	6.6 (6.1 – 7.1)	7.1 (6.4 – 7.9)	0.16	0.98
Diurnal Slope , nmol/L per hour	1510	0.8 (0.7 – 0.8)	0.8 (0.8 – 0.9)	0.8 (0.7 – 0.9)	0.55	0.06
Adjusted Cortisol Characteristics[2]						
Cortisol Awakening Response						
AUCg, nmol/L per hour	1490	18.1 (17.7 – 18.4)	18.3 (17.3– 19.3)	16.8 (15.6 – 18.1)	0.09	0.74
AUCi, nmol/L per hour	1490	2.5 (2.1 – 2.8)	2.5 (1.5 – 3.4)	2.5 (1.2 – 3.8)	0.99	0.99
Evening Cortisol, nmol/L	1525	4.9 (4.7 – 5.0)	4.5 (4.1 – 4.8)	4.2 (3.8 – 4.7)	0.004	0.04
Dexamethasone suppression test						
Cortisol suppression ratio	1452	2.4 (2.3 – 2.4)	2.5 (2.3 – 2.7)	2.3 (2.1 – 2.6)	0.71	0.46
Cortisol T7, after dexamthesone, nmol/L	1476	6.6 (6.5 - 6.8)	6.4 (5.9 – 6.9)	6.4 (5.8 – 7.1)	0.46	0.31
Diurnal Slope, nmol/L per hour	1510	0.8 (0.7 – 0.8)	0.8 (0.8 – 0.9)	0.8 (0.7 – 0.9)	0.79	0.17

For all cortisol indicators except of AUCi geometric means (95% CIs) are presented based on estimated marginal means calculated by analysis of covariance (ANCOVA). For AUCi, estimated marginal means (95% CIs) are presented. P-values are calculated by ANCOVA comparing two groups at a time. Significance is inferred at P<0.05 Nonusers are the reference group.

[1] Cortisol suppression ratio= salivary cortisol T1/salivary cortisol T7, after 0.5 mg dexamethasone. [2]Adjusted for sociodemographics (gender, age, education, North-European ancestry), sampling factors (working, weekday, time of awakening, sleep and month with more day light), comorbidity, antidepressant use (None / SSRI / TCA / other) and health indicators (smoking and physical activity).

AUCg indicates Area under the morning curve with respect to the ground; AUCi, Area under the morning curve with respect to the increase; T, timepoint; CI, Confidence Interval.

TABLE 3. Associations Between Dose and Duration of Benzodiazepine (BZD) Use as Well as Severity of BZD Dependence and Various Cortisol Indicators in a Sample of 285 BZD Users

Cortisol Indicators – Adjusted[1]	Characteristics of BZD Use					BZD Dependence (BENDEP-SRQ)						
	N	Duration of BZD Use		Daily BZD Dose		N	Problematic Use		Preoccu-pation		Lack of Compliance	
		β	*P*	β	*P*		β	*P*	β	*P*	β	*P*
Cortisol Awakening Response												
AUCg, nmol/L per hour	261	-0.031	0.64	-0.013	0.84	225	-0.005	0.95	0.057	0.39	0.048	0.49
AUCi, nmol/L per hour	261	0.010	0.88	0.048	0.47	225	-0.054	0.46	0.042	0.55	0.097	0.19
Evening cortisol												
Evening cortisol, nmol/L	267	-0.097	0.11	-0.046	0.46	231	0.037	0.58	-0.003	0.96	0.034	0.62
Dexamethasone suppression test												
Cortisol suppression ratio[2]	249	0.089	0.20	-0.084	0.22	216	0.112	0.13	0.048	0.50	-0.016	0.83
Cortisol T7, after dexamethasone, nmol/L	254	-0.147	0.03	0.028	0.68	221	-0.076	0.30	-0.011	0.87	-0.018	0.81
Diurnal Slope												
Diurnal Slope, nmol/L per hour	262	-0.036	0.59	-0.040	0.57	226	0.034	0.65	0.019	0.78	-0.064	0.39

Duration of BZD Use ranges from 1-512 months. Daily BZD dose is calculated as diazepam equivalents; mean daily doses are given, ranging from 0.05 to 105 mg (in diazepam equivalents).[1] Adjusted for sociodemographics (gender, age, education, North-European ancestry), sampling factors (working, weekday, time of awakening, sleep and month).[2] Cortisol suppression ratio= salivary cortisol T1/salivary cortisol T7, after ingestion of 0.5 mg dexamethasone.

BENDEP-SRQ indicates Benzodiazepine Dependence Self Report Questionnaire; AUCg, Area under the morning curve with respect to the ground; AUCi, Area under the morning curve with respect to the increase; β, the standardised beta coefficient by linear regression analyses

AUCg, Basal Cortisol, Cortisol Suppression Ratio and Cortisol T7 were naturally log-transformed before regression analyses.

BZD nonusers are excluded from these regression analyses.

DISCUSSION

In this study, the relationship between BZD use and various salivary cortisol measures was studied in NESDA subjects with a lifetime diagnosis of depression and/or anxiety. With the exception of slightly lower evening cortisol levels in daily and infrequent BZD users compared with non-users, the user groups did not differ on any cortisol indicators after adjustment for covariates. Dose, frequency of use, and dependence were not associated with salivary cortisol levels except of a correlation of longer duration of use with stronger cortisol suppression after dexamethasone ingestion. As the found effect sizes were small, the clinical relevance of the statistically significant findings is limited. Further, in the light of the number of tests conducted, multiple testing may have caused a type 1 error for evening cortisol in BZD users.

An explanation for the lack of consistent associations could be that BZDs inhibit the HPA axis during short-term use and that tolerance to the cortisol-suppressing effect of BZDs develops after long-term BZD treatment. Correspondingly, intervention studies that found lower cortisol levels in response to BZD administration mainly looked at short-term effects during a time period ranging from 1 day to 1 month,[3,5,6,9,13,34-37] except for a few studies with a duration of 2-3 months.[1,2,4] In contrast, chronic users were found to have similar baseline cortisol levels as nonusers, also indicating that BZDs do not maintain their cortisol-suppressing effects in long-term use.[20] As our study mainly consists of chronic users (3-year median duration of use), the lack of association between BZD use and baseline cortisol levels agrees with results from the latter study.[20]

Although tolerance is likely to develop during long-term use, an additional dosage of BZDs (on top of a regular daily dosage) still induces HPA axis inhibition. Indeed, Cowley et al.[20] found that long-term users showed similar decreases in plasma cortisol after an extra dosage of BZDs as treatment-naïve patients.[20] In related research on the therapeutic

effects of BZDs, an increased dosage of BZDs was found to increase anxiolytic effects even after more than 10 years of daily use.[38]

Along with the hypothesis of tolerance development to the cortisol suppressing effects of long-term BZD use, there are several alternative explanations that may account for discrepancies in findings. First, BZD users may have had enhanced HPA axis activity prior to the start of BZD treatment which was subsequently normalized by long-term BZD treatment. Indeed, a significantly higher percentage of daily users compared to nonusers had comorbid disorder, which has been found to be associated with increased cortisol levels in this study population.[28] Second, it might be that the joint investigation of a number of different types of BZDs with possibly opposing effects on the HPA axis has covered effects on cortisol levels.[39] We found lower diurnal slope levels and a decreased dexamethasone suppression ratio in the diazepam group and a lower AUCg in the alprazolam group compared to the oxazepam group. This may be evidence for the possibly opposing effects of the different BZDs. This corresponds to a former study that reported BZDs to have either a stimulating or an inhibiting effect on the HPA axis conditional on the alpha subunit of the GABA receptor modulated by the drugs.[39] However, as comparison groups were small in NESDA, results have to be replicated in future research. Third, stronger effects on cortisol levels may be due to higher dosages. In intervention studies higher average dosages were used than in the current study (ie, 12 mg of diazepam equivalents in intervention studies versus 6 mg in NESDA). Another explanation for basal cortisol being the only cortisol measurement differing significantly between BZD user groups might be that hippocampal mineralocorticoid receptors (MRs) are more affected by central acting BZDs than glucocorticoid receptors (GRs). Because MRs are more occupied at intermediate cortisol concentrations while GRs are not,[40] basal evening cortisol might be a probe of MR activity.[41] However, because research on GR, MR and BZDs is still limited, this assumption deserves further confirmation in future research.

Our study has some limitations. A cross-sectional analysis was done, which precludes causal inferences or differentiation between the potential explanations of the lack of group differences in salivary cortisol. Because we had to rely on subjects' self-report on BZD intake, we cannot be completely sure whether subjects were actually using the medications as prescribed and as they themselves indicated. Noncompliance with instructions of saliva collection due to the ambulatory setting could have resulted in measurement error. In addition, because time of drug intake was not recorded, acute effects of BZD use could not be assessed. Despite these limitations, our study had many strong aspects, including a large sample size with clearly distinct BZD groups primarily composed of long-term users, the inclusion of multiple cortisol measures indicative of different aspects of HPA axis activity, the investigation of various characteristics of use and the adjustment for various potential confounders.

In conclusion, we found no consistent associations between BZD use and salivary cortisol indicators within a sample primarily composed of long-term users. This finding is in line with the hypothesis that the HPA axis develops tolerance to the cortisol-suppressing effect of BZDs during chronic BZD use.

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