Initiated and discontinued Benzodiazepine Use in Relation to Autonomic Nervous System Activity

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ABSTRACT

Background: As benzodiazepines (BZDs) are used for the treatment of stress, they may affect the autonomic nervous system (ANS) which is aroused in stressful situations. Studies on the short-term effects of BZDs on the ANS are inconsistent and the effects of long-term use have hardly been studied.

Materials and Methods: In 2838 participants of the Netherlands Study of Depression and Anxiety, we examined the associations between baseline characteristics of BZD use (frequency, type, dosage, duration) and ANS measures. BZD initiators (n=85), BZD discontinuers (n=145), and chronic users (n=158) were also compared to non-users (n=1726) on absolute changes of the following ANS measures over a two-year period: heart rate [HR], respiratory sinus arrhythmia [RSA, as an indicator of PNS], and pre-ejection period [PEP, as an indicator of SNS].

Results: BZDs were used–for a median duration of two years by 442 (15.6%) of NESDA participants at baseline. At follow-up, 243 (11.5%) used BZDs. In adjusted cross-sectional analyses no associations between BZD use and ANS measures were found. During follow-up, PEP increased in BZD initiators (Cohen's d=0.23; P=0.04), but decreased in chronic users (d=0.19; P=0.03) versus non-users. No association between HR (P=0.21) and RSA (P=0.99) with BZD use was found.

Conclusion: In general, long-term BZD use does not seem to negatively affect ANS activity. The only observations were slightly increased sympathetic activity in chronic BZD users and slightly decreased sympathetic activity in new BZD users. The clinical relevance of these findings needs to be established in future research.

INTRODUCTION

The autonomic nervous system (ANS) is part of the peripheral nervous system and controls functions that are engaged in physiological homeostasis. It consists of the sympathetic (SNS) and the parasympathetic nervous system (PNS). The SNS mobilizes the fight-or-flight response; it enhances energy release and increases heart rate to prepare the body for action. The PNS stimulates "rest-and-digest" activities that occur when the body is at rest, such as digestion and salivation. In research, SNS activity can be measured through the pre-ejection period (PEP) which is a widely used, valid index of sympathetic effects on cardiac contractility.[1] PNS activity is often measured through respiratory sinus arrhythmia (RSA) which is an index of heart rate variability (HRV). Heart rate (HR) reflects both the inhibitory and augmenting control of PNS and SNS on the heart.[2]

It was recently described with cross-sectional and longitudinal data of the Netherlands Study of Depression and Anxiety (NESDA) that antidepressant medication use is associated with unfavourable effects on SNS and PNS activity.[2-4] The use of tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRI) and serotonin–norepinephrine reuptake inhibitors (SNRI) was associated with decreased HRV, the use of SNRI and TCAs was associated with decreased PEP, and the use of SSRIs was associated with increased PEP.[2-4] Consequently, it raises the important question, whether BZDs could have similar effects.

Based on animal research, two theories about the effects of BZDs on the ANS were put forward. BZDs were suggested to suppress (stress-induced) sympathetic activation by enhancing the sympathoinhibitory effects of GABA on presympathetic neurons in the paraventricular nucleus (PVN) of the hypothalamus.[5,6] These neurons are critically involved in the forebrain regulation of sympathetic outflow[5] and project to the rostral ventrolateral medulla and the spinal cord to modulate the excitability of sympathetic preganglionic neurons.[7] As GABA is localized in

discrete autonomic centers of the brain, BZDs might enhance GABAergic inhibition of sympathetic outflow by other brain structures than the PVN as well (such as nucleus ambiguous,[8] caudal ventrolateral medulla,[8] rostral ventrolateral medulla, [8] medullary raphe nuclei[9]). BZDs were also hypothesized to have vagolytic effects, meaning that they enhance the direct GABAergic inhibition of cardiac vagal neurons and the GABAergic inhibition in the nucleus tractus solitarii.[10]

Both hypotheses have been investigated in short-term intervention studies in humans with the following results. In line with the hypothesis that BZDs affect SNS activity, BZDs were generally found to suppress stress-induced increases of sympathetic activity, [11-13] except for one study where BZDs seemed to heighten sympathetic outflow[10] and another one which did not detect any SNS related effects of BZDs.[14] During rest, BZDs were either reported to decrease sympathetic tone[15-17] or not to have any effect at all. [14,18-20] Corresponding to the hypothesis that BZDs have a vagolytic effect, BZDs were commonly found to attenuate heart rate variability[10,19,21-25] and to increase HR[10,19,21,24-26]. In contrast, two studies reported heightened heart rate variability[17,27] and HR[14] after BZD administration. Thus, based on these results of experimental studies, the effects of BZDs remained unclear.

Opposite to intervention studies, observational research on the effects of BZDs on the ANS is less common. While BZDs' effects on SNS activity have not been investigated yet, cross-sectional research of our own group did not find significant differences in HRV and HR between BZD users and non-users.[2] There are several possible explanations for the discrepancy of this finding with previous experimental research that reported an attenuation of HRV caused by BZDs. As dissimilar effects of lorazepam and alprazolam on the ANS have been reported,[17] the joint investigation of different types of BZDs with possibly opposing effects might have cancelled each other out, resulting in no overall effects of BZDs on the ANS in long-term users. An alternative explanation is that only frequent BZD use and/or high BZD dosages lead to alterations of

ANS activity. Consistently, several intervention studies only found ANS alternating effects at higher dosages of BZDs[22,28] and a dose-response effect of BZDs was reported.[21,27] Finally, long-term users might develop tolerance to the effects of BZDs on the ANS so that they no longer differ from non-users. As most studies were limited to one day of testing, little can be said about this hypothesis. Only one study reported that BZDs still increases HR after seven nights of use,[28] indicating that at least for this duration of use no tolerance develops.

As the potential effects of type of BZD, dosage, duration and frequency of BZD use on the ANS have not been studied previously, additional research is needed. The current study examined the associations between several characteristics of BZD use (frequency, type of BZD, daily dosage, duration of use) and SNS and PNS measures at baseline in 2838 subjects participating in the Netherlands Study of Depression and Anxiety (NESDA). Additionally, we compared BZD initiators, BZD discontinuers and chronic users to non-users (n=2114) over a two-year period on changes in several SNS and PNS functioning parameters in order to confirm or refute our previous cross-sectional results.[2]

MATERIALS AND METHODS

Subjects

Subjects participated in the baseline assessment of the Netherlands Study of Depression and Anxiety (NESDA), a longitudinal cohort study of 2981 respondents aged 18 to 65 years.[29] Subjects were recruited from the community, general practice and specialized mental health care institutions throughout the Netherlands. The baseline interview consisted of a blood draw, a medical examination, supine rest with blood pressure recordings, psychiatric interviews, a cognitive computer task, and saliva sampling. 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.

For the cross-sectional analyses, we excluded subjects with lacking data on ANS measures (n=143). For prospective analyses, additionally, subjects who did not participate in the follow-up assessment (n=385) or those with lacking follow-up data on BZD or ANS measures were excluded (n=482). Consequently, our final sample consisted of 2838 subjects at baseline and 2114 subjects at follow-up. At baseline and follow-up subjects who reported daily or less regular BZD use in the month prior to the baseline interview were defined as "BZD users" (baseline: n=442, follow-up: n=243) and those reporting no use of BZDs in the month before the baseline interview were defined as "non-users" (baseline: n=2396, follow-up: n=1871). For the follow-up measurement, we divided subjects into "non-users" (subjects who did not use BZDs during the whole follow-up period, n=1726), "BZD initiators" (subjects who did not use BZDs at baseline, but initiated use during follow-up, n=85), "chronic users" (subjects who used BZDs at baseline and follow-up, n=158), and "BZD discontinuers" (subjects who used at baseline, but discontinued during follow-up, n=145) independent of dose and frequency of use in order to maximize group sizes.

BZD Use

As characteristics of BZD use might be associated with ANS function,[27] four indicators of BZD use were investigated at baseline: frequency of BZD use, type of BZD, daily BZD dose, and duration of BZD use. BZD use during the month prior to baseline interview was registered by observation of drug containers brought to the interview (approximately 70% of cases) or self-report. Daily and infrequent BZD users reported the type and dosage of BZD taken on an average day of use.[30] The daily BZD dose was computed according to the coding system of the Anatomical Therapeutic Code (ATC) and Defined Daily Dose (DDD) system.[31] The Mean Daily Dose was calculated by dividing individual daily doses (in milligrams) of BZDs by the DDD for the particular BZD. Frequency of use for infrequent users was taken into account when calculating the

average daily dose.[30] BZDs were classified as ATC-coded groups N05BA, N05CD, and N03AE01. The non-BZD hypnotics zopiclone and zolpidem (ATC code N05CF), were also included. For patients using BZDs other than diazepam, equivalent daily doses were calculated with conversion tables.[30,32,33] Dosages were summed when more than one BZD was used. The duration of BZD use was reported in months.

Physiological Measurements of the Autonomic Nervous System

Physiological recording was performed using the Vrije Universiteit Ambulatory Monitoring System (VU-AMS; Vrije Universiteit, Amsterdam, the Netherlands). The VU-AMS is a lightweight portable device that records electrocardiograms (ECG) and the impedance cardiogram (ICG) from 6 electrodes placed on the chests and backs of participants.[34,35] Recording was unobtrusive, and participants, who maintained full freedom of movement, tended to adjust very rapidly to this type of recording. Details on the VU-AMS recording can be found elsewhere.[2,36] In short, NESDA participants wore the VU-AMS device during most of the baseline assessments. The start of the various assessments was indicated by an event marker to divide the total recording into fixed periods (resting baseline, breaks, and test periods [interview 1, computer task, and interview 2]). Movement registration by a vertical accelerometer was used to excise periods in which participants were not stationary. Removal of breaks and non-stationary moments (about 15 minutes) left an average registration of 99.9 minutes (standard deviation [SD], 23.0 minutes). The ANS controls several aspects of cardiac function, and is therefore reflected by the following indices: HR (controlled by the balance between the PNS and SNS), respiratory sinus arrhythmia (RSA, an indicator for heart rate variability [HRV], solely controlled by the PNS), and pre-ejection period (PEP, as a measure of sympathetic control).[2] From the ECG and the ICG, interbeat interval time series and respiration signal were extracted as described elsewhere.[34,35,37] HR was derived from the interval between R-R waves in the ECG. RSA was obtained

by directly combining the electrocardiogram data with the respiration signal to obtain the variation in the interbeat intervals restricted to the typical respiratory frequency range (0.15-0.40 Hz), as described in detail elsewhere.[38] High RSA reflects high parasympathetic activity. From the ICG PEP was derived, as described in detail elsewhere.[39] Under conditions of unchanged preload and after load, the PEP is a pure measure of SNS control on the contractility of the heart, with high PEP signaling low SNS activity.[36] The mean HR, PEP, and RSA were computed for rest and test conditions at baseline and follow-up separately. As rest and test scores for HR and RSA were not significantly different, they were collapsed to a single 'test' condition for each ANS indicator to simplify analyses.[40] As PEP data during the computer task and the two interview conditions was also found to be very comparable, these data were combined to create one single PEP value per subject.[3]

Covariates

As respiration rate has often been identified as possible confounder of HRV,[41] we adjusted RSA analyses for respiration rate. Further important covariates in analyses of ANS have been identified in previous research in our study group.[47,48] Those relevant for analyses on BZDs were sociodemographic characteristics (age, gender, education), health indicators (body mass index (BMI), physical activity, alcohol use, smoking, presence of a heart disease, number of chronic illnesses, and medication (use of heart medication, frequent use of antidepressant medication including selective serotonin reuptake inhibitors [SSRIs, ATC code N06AB], tricyclic antidepressants [TCAs, ATC code N06AA], and selective serotonin and noradrenalin reuptake inhibitors [including monoamine oxidase inhibitors, nonselective N06AF, and antidepressants classified as N06AX].

Sociodemographic characteristics included age, gender, and education in years and were reported during the baseline interview. Health indicators were measured at baseline and follow-up. BMI was

calculated as weight in kilograms divided by the square of height in meters. Physical activity was measured using the International Physical Activity Questionnaire[42] and expressed as MET-minutes per week (the multiple of one's resting metabolic rate times minutes of physical activity per week). Smoking was categorized as non-smoker versus smoker. For regular alcohol use, a continuous variable was computed as mean number of alcoholic consumptions per day. Self reports were used to ascertain the presence of heart disease (including coronary disease, cardiac arrhythmia, angina pectoris, heart failure, and myocardial infarction). The number of other chronic conditions such as diabetes, stroke, and cancer was ascertained by self-report and summed into a count variable. The presence of insomnia was determined using the Insomnia Rating Scale (IRS).[43] Medication use was recorded at baseline and follow-up. Dichotomous variables for the use of heart medication were computed at both time points, scoring 'yes' if subjects frequently (daily or>50% of the time) used a medication with the following ATC codes: cardiac therapy, C01; antihypertensive drugs, C02; diuretic drugs, C03; peripheral vasodilator drugs, C04; vasoprotective drugs, C05; β-blocking agents, C07; and calcium channel blockers, C08. In addition, frequent use (daily or >50% of the time) of selective serotonin reuptake inhibitors (SSRIs, ATC code N06AB), tricyclic antidepressants (TCAs, ATC code N06AA), and serotonin and noradrenalin reuptake inhibitors (SNRI; including monoamine oxidase inhibitors, nonselective N06AF, and antidepressants classified as N06AX) was defined at either of the time points.

Statistical Analyses

Characteristics of study groups at baseline and follow-up were expressed by frequencies, means or medians, and compared using $\chi 2$ statistics (categorical variables), analyses of variance (continuous variables, normal distribution), and Kruskal-Wallis tests (continuous variables, non-Gaussian distribution).

Analyses of variance (ANOVAs) were conducted to compare non-users, infrequent users and daily users as well as the different types of BZDs at baseline on RSA, PEP, and HR. Linear regression analyses were used to assess associations between baseline characteristics of BZD use (i.e., duration of BZD use and BZD dose) and ANS indicators as continuous dependent variables. All analyses were conducted unadjusted as well as after adjustment for sociodemographic characteristics in model 1 and additional adjustment for health indicators and used medication in model 2. We did not adjust for diagnosis and severity of depression and/ or anxiety, as these diagnoses were not associated with ANS variables in previous research of our study group. Analyses of variance (ANOVAs) were conducted to compare BZD initiators, BZD discontinuers and chronic users to non users on absolute changes of RSA, PEP, and HR between baseline and follow-up in order to confirm or refute cross-sectional results by prospective data. In order to investigate changes of ANS indices between the baseline and the follow-up measurement, a change score was calculated by subtracting the baseline RSA, PEP and HR value from the follow-up value. A higher score indicated higher values at follow-up, as compared to baseline. These analyses were adjusted for above mentioned covariates in model 1 as well as for health indicators and medication use as measured at follow-up in model 2. We additionally performed sensitivity analyses after separate exclusion of the different antidepressant groups (TCA, SSRI, and SNRI users at baseline and / or follow-up), in order to make sure that our findings would not be confounded by the strong effects of antidepressant use on the ANS. Cohen's *d* (i.e., the difference in group means, divided by their pooled standard deviation) was calculated as a measure of effect size. Post-hoc tests on individual group differences were performed using the Fisher's Least Significant Difference test. Statistical significance was inferred at $P < 0.05$. All statistical analyses were conducted using SPSS version 16.0 for Windows.

RESULTS

Characteristics of the three user groups as defined at baseline are presented in Table 1. Daily and infrequent users were older than non-users (P<0.001) and had a lower education level (P<0.001). They had a higher BMI (P<0.001), more often suffered from heart disease (P<0.001) and more often had comorbid anxiety and depression (P<0.001). They also used more antidepressants (P<0.001). Daily BZD users used a higher daily BZD dosage than infrequent users (P<0.001).

Baseline Associations between BZD Use and ANS Measures

Table 2 presents group differences between daily users, infrequent users, and non-users on the PNS measure RSA, the SNS measure PEP, and HR. In the fully adjusted models, daily and infrequent users did not differ from non-users on HR (P=0.27), RSA (P=0.96), and PEP (P=0.08).

Table 3 shows associations between the ANS variables and the duration as well as the daily dosage of BZD use. In the fully adjusted models, neither duration nor dosage of BZD use was associated with any of the SNS and PNS measures. Further, we compared oxazepam, diazepam, alprazolam, temazepam, lorazepam and zopiclone users on the different ANS measures. Neither in unadjusted nor in adjusted analyses groups differed on HR, HRV and PEP (data not shown).

TABLE 1. Characteristics of the Study Groups (n=2838)

	Non-Users n=2396	Infrequent Users n=266	Daily Users n=176	*P*
Sociodemographic characteristics				
Gender, % female	66.4	70.7	64.8	0.32
Age, years	40.9 (40.4 – 41.4)	45.0 (43.4 – 46.5)	48.3 (46.4 – 46.5)	<0.001
Education level, years	12.0 (10.0 – 15.0)	11.0 (9.0 – 15.0)	10.0 (9.0 – 15.0)	<0.001
Lifestyle factors				
Body Mass Index, kg/m^2	25.4 (25.2 – 25.6)	26.2 (25.7 – 26.8)	26.7 (26.0 – 27.4)	<0.001
Physical Actvity, 1000 MET-min/week	3.1 (1.4 – 5.0)	2.8 (1.4 – 4.6)	2.6 (0.7 – 4.8)	0.01
Current smoker, %	28.9	26.7	21.6	0.10
Alcohol use, # drinks/day	0.4 (0.02 – 1.2)	0.3 (0.02 – 1.2)	0.02 (0.0 – 0.8)	<0.001
Physical and psychological health				
Heart disease, %	5.1	7.1	12.5	<0.001
Respiration rate, breaths/min	17.1 (17.0 – 17.1)	16.9 (16.8 – 17.1)	17.0 (16.8 – 17.2)	0.07
Number of chronic illnesses	1.0 (0.0 – 1.0)	1.0 (0.0 – 2.0)	1.0 (0.0 – 2.0)	<0.001
One year diagnosis, %				<0.001
Anxiety disorder only	14.7	20.3	15.9	
Depressive disorder only	15.2	18.0	16.5	
Comorbid disorder	23.3	44.4	55.1	
BAI Questionnaire	8.0 (3.0 – 16.0)	19.0 (9.0 – 25.0)	22.0 (11.0 – 31.0)	<0.001
IDS-SR Mood/Cognition Scale	6.0 (2.0 – 11.0)	10.5 (5.0 – 15.0)	13.0 (7.6 – 19.0)	<0.001
Medication Use				
Use of heart medication, %	3.3	5.6	9.7	<0.001
Frequent antidepressant use, %				<0.001
TCA	2.0	4.5	8.5	
SSRI	13.6	30.5	40.9	
SNRI	4.0	9.4	16.5	
Characteristics of BZD Use				
Duration of Use, months	NA	24.0 (5.0 – 84.0)	24.0 (5.0 – 96.0)	0.84
Dosage of BZD, mg	NA	1.0 (0.3 – 2.0)	6.3 (5.0 – 13.1)	<0.001
Long half-life, %	NA	17.3	19.9	0.49

MDD indicates Major Depressive Disorder; SSRI indicates Selective Serotonin Reuptake Inhibitor; TCA indicates Tricyclic Antidepressant; NA indicates not applicable. Means (95% confidence intervals) are given for continuous, normally distributed variables. Medians (interquartile ranges) are given for continuous, non-normally distributed variables (education, physical activity, alcohol use, number of chronic illnesses, BAI, IDS-SR, duration of BZD use, and daily dosage of BZD use). 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.

TABLE 2. Association Between ANS Measures and Frequency of BZD Use (n=2838)

	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	*P*
Characteristic	**Non-users (n=2396)**	**Infrequent Users (n=266)**	**Daily Users (n=176)**	
HR, beats/min				
Unadjusted	72.0 (71.6 – 72.4)	71.2 (70.1 – 72.4)	72.7 (71.3 – 74.1)	0.35
Model 1	71.9 (71.6 – 72.3)	71.4 (70.3 – 72.5)	73.3 (71.9 – 74.7)	0.07
Model 2	72.0 (71.6 – 72.4)	71.2 (70.1 – 72.4)	72.8 (71.4 – 74.3)	0.27
RSA, ms				
Unadjusted	45.8 (44.8 – 46.9)	39.7 (36.6 – 42.8)*	33.5 (29.7 – 37.3)*	<0.001
Model 1	45.0 (44.2 – 45.9)	42.4 (39.9 – 45.0)	40.5 (37.3 – 43.7)*	0.007
Model 2	44.5 (43.7 – 45.4)	44.3 (41.7 – 46.8)	44.6 (41.4 – 47.8)	0.96
PEP, ms				
Unadjusted	119.3 (118.6 – 120.0)	121.4 (119.2 – 123.6)	122.8 (120.1 – 125.5)*	0.02
Model 1	119.3 (118.5 – 120.0)	121.6 (119.4 – 123.8)*	123.2 (120.5 – 126.0)*	0.007
Model 2	119.4 (118.7 – 120.1)	120.9 (118.8 – 123.1)	122.0 (119.2 – 124.7)	0.08

HR indicates heart rate; RSA indicates respiratory sinus arrhythmia; PEP indicates pre-ejection period; BZD indicates benzodiazepine; CI indicates confidence interval. Model 1 was adjusted for age, gender, and education. Model 2 was additionally adjusted for physical activity, BMI, smoking, alcohol/day, number of chronic diseases, presence of heart disease, TCA, SSRI, SNRI, heart medication. The analyses of RSA were additionally adjusted for respiration rate. P-value was calculated by analysis of variance (ANOVA, P for linear trend). Significance was inferred at $P<0.05$. * indicates that the group differs significantly from the non-user group (post-hoc test, $P<0.05$).

TABLE 3. Associations Between BZD Dose and Duration of BZD Use and Various ANS Indicators in 442 BZD Users

ANS measures	Duration of Use			Daily BZD Dose		
	N	β	*P*	N	β	*P*
HR, beats/min						
Unadjusted	439	-0.075	0.12	436	0.025	0.60
Model 1	439	-0.062	0.22	436	0.046	0.34
Model 2	439	-0.077	0.14	436	0.047	0.35
RSA, ms						
Unadjusted	439	-0.119	0.01	436	-0.141	0.003
Model 1	439	0.028	0.54	436	-0.086	0.04
Model 2	439	0.053	0.22	436	-0.026	0.53
PEP, ms						
Unadjusted	439	-0.054	0.26	436	0.044	0.36
Model 1	439	-0.053	0.30	436	0.041	0.41
Model 2	439	-0.051	0.32	436	0.012	0.80

HR indicates heart rate; RSA indicates respiratory sinus arrhythmia; PEP indicates pre-ejection period; BZD indicates benzodiazepine;. Model 1 was adjusted for age, gender, and education. Model 2 was additionally adjusted for physical activity, BMI, smoking, alcohol/day, number of chronic diseases, presence of heart disease, TCA, SSRI, SNRI, heart medication. The analyses of RSA were additionally adjusted for respiration rate. *P* was calculated by linear regression analysis. Significance was inferred at P<0.05.

Prospective Associations between BZD Use and ANS Measures

Chronic users were older (*P*<0.001) and had more chronic diseases (*P*<0.001) than non-users, BZD initiators and BZD discontinuers. Chronic users had more severe anxiety than all other groups (*P*<0.001) and more severe depression than non-users and initiated users, but not than discontinued users. Further they had a lower education (*P*<0.001) than non- users and BZD discontinuers. All BZD user groups more often used antidepressants than non-users (*P*<0.001). At follow-up, the average HR across the whole sample was 72.7 beats/minute, the mean RSA 41.7 milliseconds and the mean PEP 119.2 milliseconds. Paired sampled t-tests showed a significant mean increase in HR (*P*<0.001) and a significant decrease in RSA (*P*<0.001), but no significant changes in

PEP (P = 0.88) over time within participants. The Spearman's correlation coefficients between baseline and follow-up measurements were 0.72 for HR, 0.81 for RSA, and 0.59 for PEP (all Ps<0.001). The mean HR was 72.6 for non-users, 73.8 for BZD initiators, 71.7 for BZD discontinuers and 73.5 for chronic users. The mean RSA was 41.7 for non-users, 41.7 for BZD initiators, 42.3 for BZD discontinuers and 41.3 for chronic users. Mean PEP was 119.1 for non-users, 121.8 for BZD initiators, 120.6 for BZD discontinuers and 117.4 for chronic users.

In fully adjusted analyses, BZD user groups did not differ on HR (P=0.21) and RSA (P=0.99). However, groups showed significant differences on PEP, even after adjustment for all covariates (P=0.009). BZD initiators displayed a higher increase of PEP between baseline and follow-up than non-users (Cohen's d=0.23; P=0.04). As higher PEP represents lower SNS activity, this indicates that BZD initiators have a higher decrease in SNS activity than non-users. Chronic users displayed a higher decrease in PEP than non-users (d=0.19: P=0.03), indicating that chronic users had higher increase in SNS activity than non-users. When TCA users (n=74) and SNRI users (n=168) were excluded in separate sensitivity analyses, these results did not change. When the SSRI users were excluded (n=441), group differences on PEP were not significant anymore (P=0.13) while the effect sizes of the difference between initiated users and non-users decreased (d=0.18) and the effect size of chronic users vs. non-users increased (d=0.20).

Figure 1 shows the prospective group differences of BZD initiators, BZD discontinuers, and chronic users compared to non-users on HR, RSA, and PEP after adjustment for all covariates at baseline and follow-up. Over the follow-up period, BZD initiators displayed a significantly higher increase in PEP while chronic users showed a significantly higher decrease in PEP versus non-users in post-hoc analyses.

TABLE 4. Prospective Associations Between Transitions in BZD Use and Changes in ANS Measures

	Non-Use (n=1726)	**Initiated use (n=85)**	**Discontinued use (n=145)**	**Continued use (n=158)**	
	Δ (95% CI)	Δ (95% CI)	Δ (95% CI)	Δ (95% CI)	*P*
HR, beats/min					
Unadjusted	0.5 (0.1 – 0.8)	1.1 (-0.5 – 2.7)	1.2 (-0.0 – 2.4)	2.6 (1.5 – 3.8)*	**0.005**
Model 1	0.5 (0.2 – 0.9)	0.8 (-0.8 –2.4)	1.0 (-0.2 –2.3)	2.4 (1.2 –3.6)*	**0.04**
Model 2	0.6 (0.3 – 1.0)	0.3 (-1.2 –1.9)	1.0 (-0.2 – 2.2)	1.9 (0.7 – 3.1)	0.21
RSA, ms					
Unadjusted	-2.1 (-2.8 – -1.3)	-4.0 (-7.3 – -0.6)	-1.4 (-3.9 – 1.2)	-2.3 (-4.7 – 0.1)	0.67
Model 1	-1.9 (-2.6 – -1.2)	-3.9 (-7.1 – -0.6)	-2.6 (-5.1 – - 0.0)	-3.2 (-5.7 – - 0.8)	0.52
Model 2	-2.1 (-2.8 – -1.4)	-2.7 (-5.9 – -0.6)	-2.2 (-4.7 – -0.3)	-2.3 (-4.7 – 0.2)	0.99
PEP, ms					
Unadjusted	0.7 (-0.1 – 1.4)	2.0 (-1.5 – 5.5)	-2.9 (-5.5 – -0.2)*	-5.0 (-7.5 – -2.4)*	**<0.001**
Model 1	0.4 (-0.4 – 1.1)	2.8 (-0.6 – 6.3)	-1.3 (-4.0 – 1.4)	-3.6 (-6.2 – -1.0)*	**0.008**
Model 2	0.3 (-0.5 – 1.0)	3.8 (0.5 – 7.1)*	-1.6 (-4.2 –1.0)	-2.7 (-5.2 – -0.2)*	**0.009**

HR indicates heart rate; RSA indicates respiratory sinus arrhythmia; PEP indicates pre-ejection period; BZD indicates benzodiazepine.
Model 1 was adjusted for baseline age, gender, education, physical activity, BMI, smoking, alcohol/day, number of chronic diseases, presence of heart disease, TCA, SSRI, SNRI, and heart medication. The analyses of RSA were additionally adjusted for respiration rate. Model 2 was additionally adjusted for physical activity, BMI, smoking, alcohol/day, number of chronic diseases, presence of heart disease, TCA, SSRI, SNRI, and heart medication at follow-up.
P was calculated by analysis of variance (ANOVA). Significance was inferred at P<0.05. * indicates that the group differs significantly from the non-user group (post-hoc test, *P* <0.05)

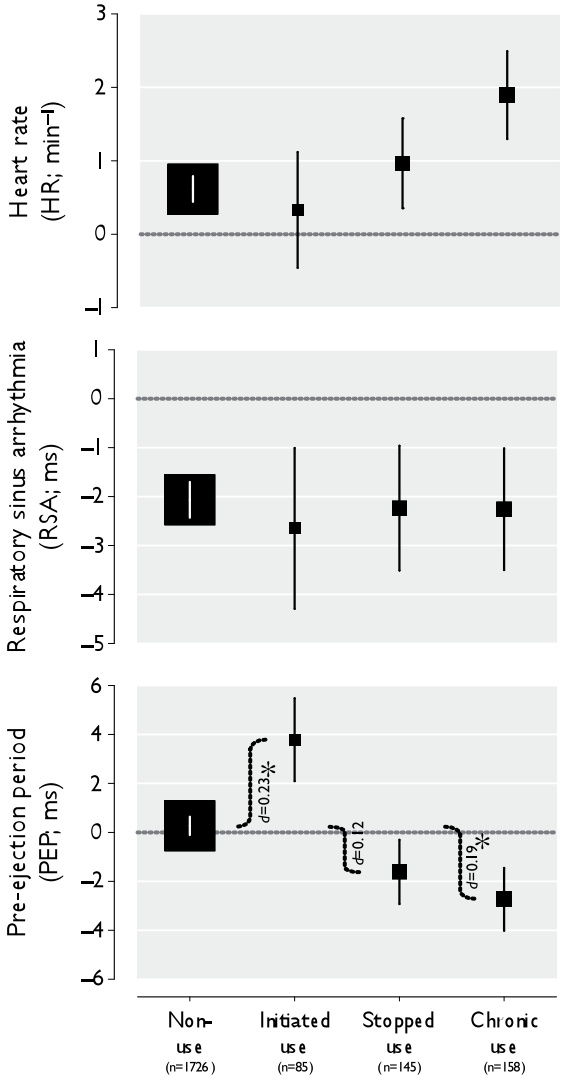

FIGURE 1. Prospective Associations Between Transitions in BZD Use and Changes in ANS Measures

DISCUSSION

In this study, possible associations between BZD use and various measures of the autonomic nervous system (ANS) were studied over a two-year follow-up period. In cross-sectional analyses, BZD use in general and characteristics of use (type, dose, frequency, and duration of use) were not associated with any of the ANS indicators. In contrast to previous research,[17] alprazolam did not have different effects on the ANS than other BZDs. In prospective analyses, BZD initiators displayed slightly lower SNS activity while BZD chronic users displayed slightly higher SNS activity. No associations between BZD use and PNS function were found. As effect sizes of the found group differences were relatively small, the clinical relevance of these findings is questionable. As the increase in PEP in BZD initiators opposed that of the decrease found in chronic users, the significant results might be chance findings considering the number of tests conducted in this study. The absence of strong effects on the ANS by BZDs is in contrast with our earlier observations for antidepressants, for which we found unfavourable effects on SNS and PNS activity.

In our cross-sectional analyses, we did not detect associations between SNS functioning and BZD use. In prospective analyses, BZD initiators showed a decrease in SNS activity versus non-users, indicating that BZD use may slightly suppress SNS functioning. This finding is in line with a number of studies that found a suppression of SNS activity (as measured by spectral power analysis,[11,12,15] norepinephrine,[16] and muscle sympathetic nerve activity[15]) after BZD administration,[11-13,15-17] but in contrast with those that reported heightened SNS activity[10] or HR[10,19,21,24-26] upon BZD intake. As in NESDA most subjects initiated BZD use longer than two months ago, the slight, sustained reduction in SNS activity in BZD initiators suggests that tolerance to BZDs SNS decreasing effect has not developed, at least not within several months. However, a suppression of SNS activity should also be reflected in a decrease of HR which was not present in our study. This complicates the interpretation of our results.

Further, chronic users displayed a higher increase in SNS activity between baseline and follow-up than non-users and also had higher absolute SNS activity values than the non-user group. This might be explained by the development of tolerance to the SNS decreasing effects of BZDs in chronic use. However, as the majority of chronic BZD users were already using for a long duration of time when the baseline measurement took place, tolerance development would have been expected much earlier. Therefore, these explanations are unlikely. As the cross-sectional and longitudinal results of the PEP analyses were quite inconsistent and the decrease of PEP was not accompanied by a decrease in HR, the group differences are difficult to explain and further research is needed to clarify the clinical relevance of our findings. When the sizeable group of SSRI users was excluded in a sensitivity analysis, the effect size of the difference between BZD initiators and non-users decreased by 20%, but the effect size of the difference between chronic users and non-users increased by 5%. As SSRIs were found to decrease sympathetic activity in previous research,[3] a small part of the SNS decrease found in BZD initiators may have been driven by concomitant SSRI use.

The absence of group differences on PNS activity in our cross-sectional and prospective analyses contrasts with the BZD induced lowered HRV and elevated HR values found in experimental research.[10,19,21-26] There are several possible explanations for these discrepancies. Tolerance may have developed to the effects of BZDs on the ANS, so that BZDs do not affect PNS activity in chronic BZD users as they do in short-term users.[21,22,24] This hypothesis is not supported by previous research which did not report tolerance development to BZDs effects on HR after a duration of seven days.[28] However, other research indicated that BZD induced HR increases went back to baseline after approximately 30 minutes, suggesting that this effect might be transient in nature.[10,24] Alternatively, NESDA mainly consists of participants using relatively low dosages of BZDs (median daily dosage of 6.0 mg diazepam equivalents) and previous research mainly found alternated ANS activity

with higher dosages.[22,28] However, some studies also found ANS effects with comparably low dosages of BZDs.[27] Further, low dosage BZD use presents the daily treatment practice so that the NESDA BZD user sample is representative of the average BZD user.

A dysregulation of the ANS can manifest itself as a reduction in HRV, an increase in HR, and heightened SNS activity. These alterations of the ANS are established risk factors for cardiovascular disease (CVD) such as coronary heart disease and acute myocardial infarction.[44-46] As BZDs are often used for long periods of time, adverse effects on the ANS may put users at a higher risk to develop CVD. This is especially true when BZDs are used in the treatment of anxiety caused by chest pain and myocardial ischemia.[47] Therefore it is reassuring that BZDs - unlike antidepressants - do not seem to affect PNS functioning in long-term users.[2] Furthermore, BZDs may even modestly decrease SNS activity. In contrast, chronic BZD use was associated with a slight increase in SNS activity and might thus be harmful, especially for patients with established CVD.

Our study has some limitations. We were not able to investigate the effects of high dosages of BZDs as the median daily dosage in NESDA was relatively low. As we had to rely on subjects' self-report on BZD intake, we cannot be sure whether subjects were actually using the medications as prescribed and as they themselves indicated. In addition, as the time of the most recent drug intake was not recorded we could not know if and how long ago the most recent BZD intake had taken place. This might have reduced the reported effects. Despite these limitations, our study had also several strengths. We were the first study to investigate the potential effects of type of BZD, dosage, duration and frequency of BZD use on the ANS. Further, we were able to investigate the effects of transitions of BZD use on the ANS over a two-year follow-up while correcting for the most important confounders. Finally, we included several aspects of ANS activity and investigated a user group representative of the average BZD

user. Therefore, our results may reflect the actual effects of BZDs on the ANS in the average BZD user.

In conclusion, long-term BZD use does not appear to have strong adverse effects on SNS or PNS activity as earlier described for some antidepressants.[2,3] Longitudinal analyses seem to suggest that relatively recent BZD initiation might slightly suppress SNS activity while chronic BZD use might slightly increase SNS activity. Whether this finding has clinical relevance needs to be established.

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