



## Association of testosterone and BDNF serum levels with craving during alcohol withdrawal



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### ABSTRACT

Preclinical and clinical studies show associations between testosterone and brain-derived neurotrophic growth factor (BDNF) serum levels. BDNF and testosterone have been independently reported to influence alcohol consumption. Therefore, we aimed to investigate a possible interplay of testosterone and BDNF contributing to alcohol dependence. Regarding possible interplay of testosterone and BDNF and the activity of the hypothalamic pituitary axis (HPA), we included cortisol serum levels in our research. We investigated testosterone and BDNF serum levels in a sample of 99 male alcohol-dependent patients during alcohol withdrawal (day 1, 7, and 14) and compared them to a healthy male control group ( $n = 17$ ). The testosterone serum levels were significantly ( $p < 0.001$ ) higher in the patients' group than in the control group and decreased significantly during alcohol withdrawal ( $p < 0.001$ ). The decrease of testosterone serum levels during alcohol withdrawal (days 1–7) was significantly associated with the BDNF serum levels (day 1:  $p = 0.008$ ). In a subgroup of patients showing high cortisol serum levels (putatively mirroring high HPA activity), we found a significant association of BDNF and testosterone as well as with alcohol craving measured by the Obsessive and Compulsive Drinking Scale (OCDS). Our data suggest a possible association of BDNF and testosterone serum levels, which may be relevant for the symptomatology of alcohol dependence. Further studies are needed to clarify our results.

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### Introduction

Alterations in testosterone and brain-derived neurotrophic factor (BDNF) serum levels have been associated with alcohol consumption. For example, Etelälahti and colleagues reported a positive association between testosterone release and alcohol consumption in alcohol-preferring rats (Etelälahti, Saarikoski, & Eriksson, 2011). Preclinical data also show higher basal testosterone levels in alcohol-preferring rats compared to non-preferring rat lines (Apter & Eriksson, 2003), and link testosterone administration with alcohol intake (Lakoza & Barkov, 1980): in clinical studies a decrease of circulating testosterone levels related to duration of drinking has

been observed, as well as a direct association between the amount of alcohol consumed per day and the amount of free testosterone serum levels (Shiels et al., 2009). Consistently, high estradiol and testosterone levels were found to be associated with alcohol consumption in boys (de Water, Braams, Crone, & Peper, 2013). Moreover, in adolescents, testosterone serum levels were reported to predict the amount of alcohol consumed in adulthood (Braams, Peper, van der Heide, Peters, & Crone, 2016). Concretely, high testosterone levels in younger males predicted high alcohol consumption in adult males, an association that the authors suggest to be attributable to neural reward response. While testosterone seems to affect alcohol drinking behavior, conversely, alcohol consumption is known to affect testosterone secretion (Ruusa & Bergman, 1996) in a dose-dependent fashion: in males, intake of lower ethanol doses (0.5 g/kg) was reported to increase testosterone levels 2 h after ingestion (Sarkola & Eriksson, 2003), whereas intake of higher

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ethanol dosages (1.5 g/kg) was reported to reduce testosterone release 10 h after ingestion (Välämäki et al., 1990).

BDNF serum levels were frequently reported to be decreased in alcohol-dependent patients compared to healthy controls (Huang et al., 2011; Zanardini et al., 2011). Moreover, high BDNF serum levels have been associated with withdrawal intensity during early alcohol withdrawal (Heberlein, Muschler, Wilhelm, et al., 2010; Huang et al., 2008). Further results suggest that BDNF serum levels may even be a possible marker of alcohol relapse probability. For example, Costa and colleagues presented data that indicate that high BDNF serum levels reduce the risk of relapse in alcohol-dependent patients (Costa, Girard, Dalmay, & Malauzat, 2011). Consistent with these results, we found an association between drinking history and promoter methylation of the BDNF gene in our own data: we found a negative association of BDNF IV-promoter methylation and duration of abstinence before relapse, which supports a possible protective effect of high BDNF expression regarding alcohol relapse (Heberlein et al., 2015). Further support for a possible association comes from animal data, which demonstrate that prolonged alcohol consumption results in a decrease of central BDNF expression (McGough et al., 2004; Raivio, Miettinen, & Kiiänmaa, 2014), suggesting that the decrease of central BDNF expression resulting from alcohol consumption may promote further alcohol consumption.

Considering these study results, the question arises of a relevant interplay of testosterone and BDNF regarding alcohol intake. Indeed, there is research that supports a possible association between the neurotrophic growth factor BDNF and testosterone. For example, testosterone-dependent increase of hippocampal neuronal growth and testosterone-induced increase of synaptic plasticity were associated with concomitant BDNF expression (Atwi, McMahon, Scharfman, & MacLusky, 2016). Moreover, testosterone administration was shown to decrease cerebral ischemic infarct volume in rats by increasing BDNF expression in the affected area (Fanaei et al., 2014). Nevertheless, the concrete mechanisms of this possible interplay have not yet been elucidated. However, study results point toward an interaction between cytokine release, sex hormones, and neurotrophic growth factors. For example, Xu and colleagues reported that increased survival of bulbocavernosus motoneurons by testosterone was blocked by tropomyosin receptor kinase B (TrkB), which is the high-affinity receptor for BDNF antagonists (Xu, Gingras, Bengston, Di Marco, & Forger, 2001). Conversely, there are study results which demonstrate interplay between cytokine release and the TrkB receptor, suggesting that neurotransmission via the TrkB may link inflammation, sex hormones, and neurotrophic growth factors (Zhang, Yao, & Hashimoto, 2016). Consistent with these reports are study results which suggest that the activity of the hypothalamic pituitary axis (HPA) (Moonat & Pandey, 2012) may be a relevant factor, which supports a possible association of BDNF and testosterone. Indeed, there are preclinical study results that demonstrate the influence of HPA activity on BDNF as well as on testosterone release. For example, basal HPA activity was negatively associated with cerebral BDNF expression. Moreover, a negative association between *de novo* synthesis of BDNF and the adaption of the stress response was reported (Maghsoudi et al., 2014; Naert, Maurice, Tapia-Arancibia, & Givalois, 2007). Regarding a possible influence of HPA activity on testosterone levels, Toufexis and colleagues reported that physiological doses of dihydrotestosterone decreased basal levels of serum cortisol in male and female macaques and also decreased corticotrophin-releasing factor- (CRF) induced activation in male macaques (Toufexis & Wilson, 2012).

Study results also suggest a link between testosterone-related behavioral impulsivity (Cooper, Goings, Kim, & Wood, 2014) and

HPA activity (Mehta, Welker, Zilioli, & Carré, 2015). Mehta et al. (2015) reported that risk taking and testosterone serum levels were positively associated in male and female probands when HPA activity was low, suggesting a link between impulsivity, testosterone release, and HPA activity. Partly closing the gap between alterations of testosterone levels, HPA activity, and alcohol consumption, prenatal exposure to ethanol was reported to reduce testosterone's effect on the HPA (Lan, Hellemans, Ellis, Viau, & Weinberg, 2009). Moreover, corticosterone replacement therapy was reported to result in increased alcohol consumption in adrenalectomized alcohol-preferring male rats, implicating a direct corticosterone effect on alcohol consumption in vulnerable populations (Fahlke & Eriksson, 2000).

These results suggest an association of high testosterone release, decreased HPA activity, increased BDNF expression, and behavioral traits, which may influence impulsive behavior such as alcohol consumption. Strengthening the hypothesis of a multilateral association of sex hormones, HPA activity, and BDNF, Franklin and colleagues reported that stress increased or decreased BDNF release, depending on the availability of sex hormones (Franklin & Perrot-Sinal, 2006).

The aim of our study was to investigate alterations of testosterone serum levels due to intoxication and during withdrawal in alcohol-dependent patients. Moreover, we focused on possible associations between the symptomatology of alcohol withdrawal, testosterone, and BDNF. Regarding the possible relevance of the activity of the HPA axis, we also investigated a possible association between testosterone and BDNF serum levels in subgroups of patients displaying high versus low cortisol levels.

## Materials and methods

The present study was part of a large prospective research project (Studies in Neuroendocrinology and Neurogenetics in Alcoholism [NENA]) (Heberlein, Muschler, Lenz, et al., 2010) that was approved by the local Ethics Committee of the Friedrich-Alexander University Erlangen-Nürnberg. In this sample we had already investigated alterations of BDNF serum levels during alcohol withdrawal (Heberlein, Muschler, Wilhelm, et al., 2010). The investigation was conducted in accordance with the Declaration of Helsinki. Each participant gave written informed consent.

In total, we investigated the testosterone and BDNF serum levels of 99 male patients who suffered from alcohol dependence, according to ICD-10 and DSM-IV. All patients were admitted for detoxification treatment (Klinik für Psychiatrie, Psychotherapie und Psychosomatik, Bezirksklinikum Obermain, Kutzberg, Germany). The patients' group consisted of smokers and non-smokers (10 non-smokers, 81 smokers, 6 former smokers, 2 unknown). Table 1 shows the demographic data of the patients' group. Patients with concomitant psychiatric illnesses, substance abuse apart from alcohol or nicotine, existence of severe somatic illnesses (in particular patients suffering from any type of cancer), known autoimmune diseases, or known HPA axis deregulations were not enrolled in the study. In addition, patients with a positive history of cerebral damage (e.g., ischemia or cerebral hemorrhage) were excluded. All patients underwent a detailed physical examination, routine laboratory testing, and urine drug screening. Patients received carbamazepine and/or clomethiazole in order to treat alcohol-withdrawal symptomatology. Dosages were adjusted to the individual severity of alcohol withdrawal. Blood samples were taken before the patients took their morning medication.

Breath alcohol concentration was measured on admission and during alcohol withdrawal using the alcohol breath analyzer (Draeger, Dietikon, CH). Additional data such as age, body mass index (BMI), years of drinking, and daily intake of alcohol in grams

**Table 1**

Demographic data of the patients' and the control group, means, and standard deviation (SD) of the test results.

	Patients' group	Control group	<i>p</i>	Early abstinent ( <i>n</i> = 24)	Positive breath alcohol ( <i>n</i> = 57)	<i>p</i>
Age	42.22 ± 7.83	44.41 ± 9.63	n.s.	42.50 ± 10.64	43.96 ± 7.13	n.s.
BMI	24.62 ± 3.60	26.44 ± 4.58	n.s.	25.01 ± 5.49	25.07 ± 4.02	n.s.
Years of drinking (y)	9.42 ± 7.67	n.a.	n.a.	8.95 ± 9.26	8.74 ± 7.22	n.s.
Daily intake (g)	194.90 ± 83.42	n.a.	n.a.	171.55 ± 81.90	202.02 ± 88.63	n.s.
BDNF (pg/mL)	Day 1: 641.67 ± 511.71 Day 7: 556.33 ± 400.92 Day 14: 651.74 ± 519.40	728.84 ± 383.38	n.s.	Day 1: 478.55 ± 350.14 Day 7: 527.98 ± 439.09 Day 14: 503.10 ± 464.19	Day 1: 706.24 ± 512.05 Day 7: 551.71 ± 420.39 Day 14: 691.15 ± 511.37	Day 1: 0.044 Day 7: n.s. Day 14: n.s.
Testosterone (ng/mL)	Day 1: 10.05 ± 11.42 Day 7: 6.06 ± 12.69 Day 14: 5.64 ± 8.69	5.11 ± 5.42	Day 1: <0.001 Day 7: 0.001 Day 14: 0.003	Day 1: 14.19 ± 16.88 Day 7: 13.64 ± 19.04 Day 14: 9.87 ± 6.16	Day 1: 11.02 ± 6.68 Day 7: 9.64 ± 7.44 Day 14: 8.41 ± 3.86	Day 1: n.s. Day 7: n.s. Day 14: n.s.
Cortisol (nmol/L)	Day 1: 281.85 ± 133.98 Day 7: 204.16 ± 101.22 Day 14: 261.82 ± 182.02	183.00 ± 87.66	Day 1: 0.002 Day 7: n.s. Day 14: 0.003	Day 1: 252.55 ± 145.42 Day 7: 164.39 ± 91.74 Day 14: 234.03 ± 100.13	Day 1: 276.38 ± 124.58 Day 7: 221.42 ± 105.01 Day 14: 261.51 ± 161.73	Day 1: n.s. Day 7: 0.035 Day 14: n.s.
OCDS total score	Day 1: 19.80 ± 6.70 Day 7: 10.92 ± 7.42 Day 14: 9.88 ± 6.71	n.a.	n.a.	Day 1: 18.42 ± 6.33 Day 7: 8.58 ± 7.083 Day 14: 8.90 ± 5.41	Day 1: 19.81 ± 6.96 Day 7: 11.43 ± 7.12 Day 14: 10.54 ± 7.01	Day 1: n.s. Day 7: n.s. Day 14: n.s.
PACS score	Day 1: 16.15 ± 7.56 Day 7: 6.27 ± 5.78 Day 14: 5.07 ± 6.17	n.a.	n.a.	Day 1: 16.88 ± 6.3 Day 7: 4.73 ± 4.52 Day 14: 3.75 ± 4.089	Day 1: 15.73 ± 7.75 Day 7: 6.69 ± 5.95 Day 14: 5.29 ± 6.48	Day 1: n.s. Day 7: n.s. Day 14: n.s.
CIWA	Day 1: 15.64 ± 4.23 Day 7: 12.84 ± 2.75 Day 14: 12.33 ± 2.35	n.a.	n.a.	Day 1: 14.04 ± 3.5 Day 7: 12.73 ± 2.35 Day 14: 12.37 ± 2.45	Day 1: 15.98 ± 4.27 Day 7: 12.93 ± 2.87 Day 14: 12.13 ± 2.41	Day 1: 0.045 Day 7: n.s. Day 14: n.s.
BDI	Day 1: 17.40 ± 8.75 Day 7: 8.23 ± 7.60 Day 14: 5.97 ± 7.26	3.00 ± 3.77	Day 1: <0.001 Day 7: 0.006 Day 14: n.s.	Day 1: 17.96 ± 10.08 Day 7: 9.27 ± 8.39 Day 14: 4.80 ± 6.92	Day 1: 16.02 ± 7.92 Day 7: 7.47 ± 7.12 Day 14: 6.23 ± 7.33	Day 1: n.s. Day 7: n.s. Day 14: n.s.
STAI-I	Day 1: 48.78 ± 12.59 Day 7: 37.64 ± 10.96 Day 14: 37.70 ± 11.96	31.47 ± 5.34	Day 1: <0.001 Day 7: 0.043 Day 14: 0.006	Day 1: 46.96 ± 9.544 Day 7: 35.09 ± 12.9 Day 14: 35.0 ± 12.10	Day 1: 49.05 ± 13.36 Day 7: 38.27 ± 10.64 Day 14: 38.55 ± 12.93	Day 1: n.s. Day 7: n.s. Day 14: n.s.
STAI-II	Day 1: 47.86 ± 10.83	30.65 ± 6.94	Day 1: <0.001	Day 1: 49.0 ± 11.32	Day 1: 47.38 ± 10.89	Day 1: n.s.
SESA	Day 1: 48.92 ± 18.05	n.a.	n.a.	Day 1: 44.70 ± 18.17	Day 1: 49.94 ± 20.36	

n.s.: not significant n.a.: not available.

were obtained by interview. Data regarding affective symptoms were collected by the Beck's Depression Inventory (BDI) (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961) and the State and Trait Anxiety Inventory (STAI-I and STAI-II) (Spielberger, Gorsuch, & Lushene, 1970). Intensity of alcohol craving was measured by the Obsessive and Compulsive Drinking Scale (OCDS) (Anton, Moak, & Latham, 1995). Severity of alcohol withdrawal was measured by the Clinical Institute Withdrawal Assessment for Alcohol (CIWA-A) (Stuppaek et al., 1994). The severity of alcohol dependence was assessed by the Severity of Alcohol Dependence (SESA) questionnaire and its subscales (John, Hapke, & Rumpf, 2003). The subscales of the SESA questionnaire measure narrowing of the drinking repertoire (SESA-XE), somatic withdrawal symptoms (SESA-XK), alcohol consumption to avoid withdrawal symptoms (SESA-XV), psychological withdrawal symptoms (craving) (SESA-XP), increase of tolerance (SESA-XT), extreme increase of tolerance (SESA-XTE), and decrease of tolerance (SESA-XTU).

Psychometric measurements of affective symptoms of alcohol withdrawal were taken once a day on day 1, day 7, and day 14 between 8:00 and 10:00 AM, immediately after blood withdrawal. All blood samples (in the patients' group and in the control group) were taken between 8:00 and 10:00 AM.

As a control group, we enrolled randomly selected healthy males (*n* = 17) who did not suffer from any current somatic disease. Controls were screened for alcohol dependence and abuse using the CAGE questionnaire (Mayfield, McLeod, & Hall, 1974) and the alcohol use disorder identification test – alcohol consumption questions (AUDIT-C) (Saunders, Aasland, Babor, de la Fuente, & Grant, 1993). A score of 0 points in the CAGE questionnaire and a score below 3 points in the AUDIT-C were required for inclusion in the control group. Controls were negative for alcohol abuse, alcohol dependence, and any other Axis 1 diagnosis according to ICD-10 or DSM-IV. Controls received no psychopharmacological treatment.

The control group consisted of actual smokers (3 persons) and former smokers (14 persons).

The BDNF, the testosterone, and the cortisol serum levels were assessed using enzyme-linked immunosorbent assay (ELISA) (DY248, KGE010, KA1885, R&D Systems, Wiesbaden-Nordenstadt, Germany). All the assays were performed according to the manufacturer's directions. The lower thresholds of determination were 23.4 pg/mL (BDNF), 0.041 ng/mL (testosterone), and 0.456 ng/mL (cortisol). The assay ranges were 23.4–1500 pg/mL (BDNF), 0–10 ng/mL (testosterone), and 0.66–20 ng/mL (cortisol). The interassay coefficients of variation were 5.0 and 8.7%. Besides BDNF, testosterone, and cortisol serum levels, we assessed liver enzyme levels, creatinine, urea blood count, and c-reactive peptide levels in all patients and controls.

### Statistical analysis

The testosterone serum levels were not normally distributed according to the Kolmogorov–Smirnov test. Log- and In-transformation did not transform the testosterone serum levels into normal distribution; therefore, non-parametric statistics were applied. In order to assess the alterations of testosterone serum levels throughout alcohol withdrawal, three difference values were calculated, which mirror the alterations of testosterone serum levels from day 1 to day 7, from day 7 to day 14, and from day 1 to day 14. Moreover, in order to assess a possible impact of HPA activity, a median split was calculated to diverge patients with high (subgroup A) and low (subgroup B) cortisol serum levels throughout alcohol withdrawal.

Correlations were calculated by the Spearman's correlation coefficient. Mean differences between the testosterone serum levels of the alcohol-dependent patients on day 1, 7, and 14 were calculated by Friedman test. Group-to-group differences

between the healthy control group and the patients' group were calculated by the Mann–Whitney  $U$  test. Significance level was set to  $\alpha = 0.05$ .

Data were analyzed by SPSS 20 (SPSS Inc., Chicago, IL). Graphs were developed by Graph Pad Prism™ 5.0 (Graph Pad Software, Inc., San Diego, CA).

## Results

### Demographic data of the patients' group

Data concerning the demographic characteristics of both groups and data concerning the history and amount of alcohol consumption in the patients' group are shown in Table 1. There was no association between the testosterone serum levels and the BMI of the participants. Testosterone levels on day 14 of alcohol withdrawal were significantly associated with the age of the participants ( $\rho = -0.265$ ,  $p = 0.012$ ).

### Association of testosterone with liver enzyme levels and blood count

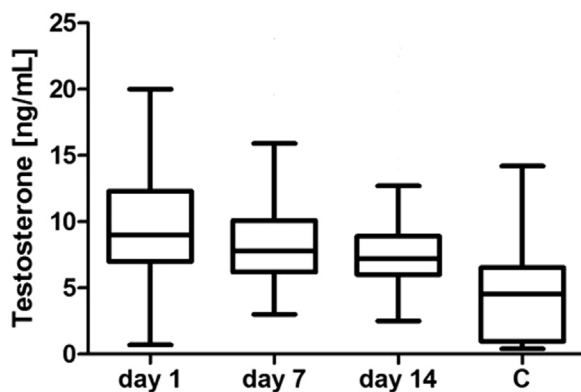
The testosterone serum levels were not associated with liver enzyme levels during alcohol withdrawal (data not shown). There was no association with leukocyte count, thrombocyte count, or the testosterone serum levels during alcohol withdrawal (data not shown).

### Association of testosterone serum levels and alcohol withdrawal medication

There was a significant positive association between clome-thiazole dosages and testosterone serum levels on day 1 of alcohol withdrawal in patients showing positive breath alcohol concentrations on admission ( $\rho = 0.358$ ,  $p = 0.010$ ). In subgroup A, testosterone serum levels on day 1 were significantly associated with clomethiazole dosage as well ( $\rho = 0.588$ ,  $p < 0.001$ ).

### Testosterone serum levels in alcohol-dependent patients compared with healthy controls

Serum levels of testosterone were significantly higher in the alcohol-dependent patients compared with healthy controls (day 1:  $U = -3.652$ ,  $p < 0.001$ , day 7:  $U = 3.238$ ,  $p = 0.001$ , day 14:  $U = 3.019$ ,  $p = 0.003$ ; see Fig. 1). Testosterone serum levels



**Fig. 1.** Testosterone serum levels during alcohol withdrawal (day 1, day 7, day 14) compared with controls (C). The testosterone serum levels of the alcohol-dependent patients decrease on day 1, day 7, and day 14 of alcohol withdrawal. Testosterone serum levels are higher than testosterone serum levels of the healthy control group (C).

decreased significantly during alcohol withdrawal ( $\chi^2 = 32.23$ ,  $p < 0.001$ , see Fig. 1).

The BDNF serum levels of the experimental group were not significantly altered compared with the levels of the healthy control group (Heberlein, Muschler, Wilhelm, et al., 2010). The BDNF serum level decreased slightly but not significantly during alcohol withdrawal (data not shown).

### Association between testosterone serum levels and alcohol consumption

#### Association with breath alcohol concentration

There was no association between breath alcohol concentration and the BDNF, cortisol, and testosterone serum levels in the entire patients' group.

There was a significant trend toward higher BDNF serum levels in patients showing positive alcohol breath levels at admission ( $n = 57$ ) compared to early abstinent patients ( $n = 24$ ,  $p = 0.044$ ). Apart from that, there were no significant differences regarding psychometrics and serum levels between early abstinent and alcohol-intoxicated patients (see Table 1 for details).

In those patients showing higher HPA activity (subgroup A, 29 patients), the testosterone serum levels were significantly associated with breath alcohol concentration on day 1 of alcohol withdrawal ( $\rho = -0.508$ ,  $p = 0.005$ ). Moreover, the decrease of testosterone serum levels from day 1 to day 7 was significantly associated with breath alcohol concentration ( $\rho = 0.526$ ,  $p = 0.004$ ).

#### Association with anamnesis of alcohol consumption

There was no association between the duration of alcohol consumption or the daily alcohol intake and the testosterone serum levels in the entire patients' group. Nevertheless, in the group of patients showing high HPA activity (subgroup A), the testosterone serum levels on day 14 were significantly associated with the duration of self-reported alcohol consumption ( $\rho = -0.507$ ,  $p = 0.001$ ).

#### Association of testosterone, BDNF, and cortisol serum levels

Testosterone serum levels were significantly different in subgroup A and subgroup B: testosterone serum levels were significantly increased in those patients showing higher cortisol serum levels on day 1 (Mann–Whitney  $U Z = -2.58$ ,  $p = 0.010$ ).

Testosterone serum levels were significantly associated with cortisol serum levels on day 1 ( $\rho = 0.243$ ,  $p = 0.017$ ) and day 14 of alcohol withdrawal ( $\rho = 0.366$ ,  $p < 0.001$ ).

The decrease of testosterone serum levels from day 1 to day 7 of alcohol withdrawal was significantly associated with the BDNF serum levels on day 1 ( $\rho = -0.268$ ,  $p = 0.008$ ).

#### Association of testosterone serum levels and the severity of alcohol dependence

The decrease of testosterone serum levels from day 1 to day 14 was significantly associated with the XTU subscale of the SESA scale ( $\rho = 0.227$ ,  $p = 0.026$ ). There was an association neither with the further SESA subscales nor with the total value of the SESA scale (data not shown).

#### Association of testosterone serum levels with the symptomatology of alcohol withdrawal

The decrease of the testosterone serum levels from day 1 to day 7 was significantly associated with alcohol craving measured by the OCDS ( $\rho = 0.248$ ,  $p = 0.014$ ) on day 7 and with the compulsive subscale of the OCDS on day 7 ( $\rho = 0.324$ ,  $p = 0.001$ ).

Moreover, in subgroup A the decrease of testosterone serum levels from day 1 to day 7 and from day 7 to day 14 was significantly associated with BDNF serum levels ( $\rho = -0.369$ ,  $p = 0.013$ ) and with compulsive alcohol craving on day 7 of alcohol withdrawal ( $\rho = 0.417$ ,  $p = 0.004$ ). Conversely, the BDNF serum levels showed a significant association with the OCDS total score ( $\rho = 0.429$ ,  $p = 0.003$ ), the obsessive subscale ( $\rho = 0.352$ ,  $p = 0.018$ ), and the compulsive subscale ( $\rho = 0.383$ ,  $p = 0.009$ ) in this subgroup on day 1.

There was no association between the testosterone serum levels and the severity of the alcohol withdrawal syndrome measured by the CIWA score (data not shown). There was no significant association between the testosterone serum levels, the decrease of the testosterone serum levels, and the BDI score or the STAI-I/STAI-II scores (data not shown).

## Discussion

In this study, we investigated a possible link between alterations in testosterone, BDNF, and cortisol serum levels and alcohol consumption in a sample of male alcohol-dependent patients.

While study results typically demonstrate a decrease of testosterone levels due to chronic alcohol consumption (Maneesh, Dutta, Chakrabarti, & Vasudevan, 2006), higher testosterone levels have been reported in male alcohol-dependent patients in the first 3 months of abstinence (Hasselblatt, Krieg-Hartig, Hüfner, Halaris, & Ehrenreich, 2003). Consistent with such reports, we found higher testosterone serum levels in the patients' group compared to the healthy control group. High testosterone levels could be explained by dysregulation of testosterone release due to alcohol withdrawal. Consistently, we found high variations of testosterone serum levels in our study (see Table 1 for details) as well as a positive association between cortisol serum levels and testosterone serum levels on day 1 and day 14 of alcohol withdrawal.

We also found a positive association between the clomethiazole dosages and the testosterone serum levels. This indicates that the high testosterone serum levels observed in our sample may at least partly be explained by alcohol-withdrawal medication.

In agreement with study results supporting a suppressive effect of alcohol consumption on testosterone serum levels (Forquer, Hashimoto, Roberts, & Wiren, 2011), we found a negative association between the testosterone serum levels and initial breath alcohol concentration in subgroup A (those patients showing high HPA activity). Furthermore, we found a negative association between the testosterone serum levels and drinking history after early alcohol withdrawal (on day 14) as well as a positive association between the decrease of testosterone serum levels from day 1 to day 14 of alcohol withdrawal and initial alcohol intoxication in subgroup A, which is consistent with study results reporting decreased testosterone serum levels due to chronic alcohol consumption (Maneesh et al., 2006).

Regarding the symptomatology of alcohol withdrawal, our data show a positive association between the decline of testosterone serum levels during alcohol withdrawal, the BDNF serum levels, and the intensity of alcohol craving in early alcohol withdrawal. We also found a negative association between the decrease of testosterone serum levels and the BDNF serum levels, which were positively associated with the intensity of alcohol craving measured by the OCDS in subgroup A of our sample. These results suggest that there may be a relevant association between HPA activity, testosterone, and BDNF levels as reported before by some authors (Franklin, Zou, Yu, & Costello, 2006). Our results support the assumption that HPA activation is involved in the interplay between testosterone and BDNF.

Earlier study results showed negative associations between HPA activity, testosterone (Mehta et al., 2015), and BDNF (Franklin & Perrot-Sinal, 2006) release, as well as positive associations between testosterone and BDNF (Fanaei et al., 2014). Such results indicate that high HPA activity interferes with neuroregeneration and neuroneogenesis (Allen, Purves-Tyson, Fung, & Shannon Weickert, 2015), which are typically stressed following loss of hippocampal volume in depressive disorders (Yun et al., 2016). Moreover, animal data suggest that corticosteroids trigger alcohol consumption in vulnerable populations (Fahlke & Eriksson, 2000). We found a negative association between the decrease of the testosterone serum levels during alcohol withdrawal and BDNF serum levels, a result that does not fit easily with a possible positive association of BDNF and testosterone reported earlier (Yun et al., 2016). It is likely that alcohol withdrawal and alcohol-withdrawal medication may explain the results presented here. Indeed, there are preclinical study results that support the hypothesis that ethanol consumption may alter the relationship between testosterone and the HPA (Zhang et al., 2016). Therefore, it seems likely that the alcohol craving associated with alcohol withdrawal is explained by the association of BDNF and testosterone. Further studies will be necessary to clarify the implications of the associations found in our sample.

Although our data are not sufficient to allow causal conclusions, they support earlier research which suggests an interplay of sex hormones and BDNF (Fanaei et al., 2014), possibly relevant for the symptomatology of alcohol dependence. Concretely, our data are consistent with earlier results presented by our group, which demonstrate that BDNF is associated with the symptomatology of alcohol withdrawal and alcohol relapse (Heberlein et al., 2014, 2015). The results obtained in this study regarding a possible association between testosterone serum levels and BDNF serum levels supplement earlier results of our group that show a negative association between BDNF serum levels and serum levels of the cytokine tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ). Taking into account animal studies that demonstrate negative associations between testosterone and TNF- $\alpha$  (Bini et al., 2015), one could speculate about a complex interplay of different peptides such as cytokines, sex hormones, etc., and BDNF, which may contribute to the symptomatology of alcohol dependence.

Our study suffers from several limitations that constrain the interpretability of our results. One major limitation is due to the non-linearity of our testosterone data. This prompted us to use non-parametric statistics instead of variance analysis, which would have allowed the inclusion of possible covariates. Moreover, our samples were heterogeneous regarding smoking status, which may have influenced our results. A further restriction is the correlative character of our research as well as the single daily measurement of the testosterone and BDNF serum levels. A prospective study setting measuring cortisol, BDNF, and testosterone throughout the day and investigating more clearly the association between stress regulation and BDNF-testosterone interaction as suggested by our data would clearly have improved the clarity of our results.

Summing up, our study supports earlier research regarding a possible interaction between testosterone and BDNF. Moreover, it enriches current knowledge by supporting the hypothesis of a possible interplay between testosterone and BDNF, presumably dependent on HPA activity. According to our results, the decrease of testosterone release during alcohol withdrawal may underlie alcohol craving by interaction with BDNF expression. Therefore, it seems likely that testosterone contributes to core symptoms of alcohol dependence, such as alcohol craving. Further research is warranted in order to unravel the causalities of the associations observed. It is necessary to regard the various limitations of our study as well as its correlative character in order to confirm our results.

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