

CONTROLLED STUDY ON THE COMBINED EFFECT OF ALCOHOL AND TOBACCO SMOKING ON TESTOSTERONE IN ALCOHOL-DEPENDENT MEN

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Abstract — **Aims:** The present study examined the association between pre-treatment drinking and smoking parameters and plasma testosterone levels before and after alcohol withdrawal. **Methods:** A total of 51 alcohol-dependent men and 43 age-matched healthy men were investigated. In alcoholics, free testosterone in plasma was measured on the day of admission, after detoxification and after 6 weeks of sobriety. **Results:** While the testosterone level of alcoholic men did not differ from healthy controls at the onset of withdrawal, it was significantly higher for the alcoholics after 6 weeks of sobriety than for the healthy controls. Higher alcohol consumption and higher tobacco use before detoxification led to higher levels of testosterone concentration before and after withdrawal. **Conclusions:** The effect of alcohol and tobacco is cumulative, with higher levels of alcohol and tobacco consumption being associated with higher levels of testosterone before and after alcohol withdrawal.

INTRODUCTION

Alcohol intake affects the activity of the hypothalamic–pituitary–adrenal (HPA) axis (Heinz *et al.*, 1995, Walter *et al.*, 2006) and the hypothalamo–pituitary–gonadal (HPG) axis (Adler, 1992, Schiavi *et al.*, 1995). Though the underlying mechanisms have not been completely identified, animal and laboratory studies indicate that alcohol suppresses the activity of the HPG axis by inhibiting the secretion of hypothalamic–gonadotropin-releasing hormone (GnRH) and/or pituitary luteinizing hormone (LH) (Hiney and Dees, 1991, Uddin *et al.*, 1994). Additionally, alcohol exerts its harmful effect directly on the testes by reducing the testicular biosynthesis of testosterone (Välimäki *et al.*, 1990, Orpana *et al.*, 1990, Adams *et al.*, 1997).

It was found that nicotine (the main bioactive biochemical substance in tobacco) inhibited steroidogenesis in mouse Leydig cells (Patterson *et al.*, 1990). The chronic treatment with alcohol and nicotine has been reported to cause decrease in fertilization ability in male animals (Dhawan and Sharma, 2002). Furthermore, animal studies indicate that not only the direct effects of nicotine on the sex steroids synthesis but also the indirect effect of an increased corticosteroid secretion plays a central role in the effect of drug on testicular steroid synthesis in the male rat fetus (Sarasin *et al.*, 2003).

In naturalistic studies, chronic alcohol intake reduces plasma testosterone levels in men (Castilla-Garcia, 1987). Interestingly, even without alcohol consumption, plasma testosterone levels decrease in dependent men after experimental exposure to the sight and smell of alcohol (Meyer and Dolinsky, 1990). In non-cirrhotic alcoholic men, testosterone concentrations increase during withdrawal and return to normal limits after three weeks of abstinence (Heinz *et al.*, 1995, Ruusa *et al.*, 1997). During alcohol withdrawal, lower plasma testosterone levels seem to be associated with more

neurotic symptoms while higher hormone levels are related to a history of seizures (Ruusa and Bergman, 1996). Though a causal relationship has not yet been established, there is some evidence for an association between plasma testosterone levels and alcohol withdrawal symptoms (Ruusa and Bergman, 1996).

While there have been several studies investigating the effects of alcohol, little is known about the influence of tobacco smoking on sex steroids in men. It was found that nicotine inhibits pulsatile LH secretion in males, and that this effect disappears within one week after quitting cigarette smoking (Funabashi *et al.*, 2005). In healthy men, testosterone levels were negatively correlated with alcohol intake (Muller *et al.*, 2003), and mainly positively correlated with cigarette smoking (Tamimi *et al.*, 2001, Svartberg *et al.*, 2003).

To the best of our knowledge, there has been no study investigating the combined effects of alcohol and tobacco smoking on plasma testosterone levels in alcoholics. This report is the first one to examine the impact of drinking and smoking in alcohol-dependent men on the course of plasma testosterone concentrations during withdrawal.

METHODS

All data were collected as part of the Würzburg Addiction-Research Association Study. The Ethics Committee of the University of Würzburg and the Institutional Review Board of the Department of Psychiatry vetted the study protocol and gave approval for the present study. Written informed consent was obtained from each participant before inclusion.

Sample and setting

The sample consisted of 51 male inpatients on the basis of consecutive admissions to the Alcohol Dependence Treatment Unit of the Psychiatric University Hospital Würzburg (Germany). Eligible for participation were all patients with (i) current history of alcohol dependence according to ICD-10 criteria, (ii) verified maintenance of abstinence during

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their stay in the hospital, and (iii) urine toxicology screens negative for benzodiazepines, cannabinoids, barbiturates, opiates, cocaine, and amphetamines. Criteria for exclusion included: (i) evidence of severe medical or neurological conditions (e.g. cerebral, renal, thyroid, or cardiac disease), (ii) history of cirrhosis or laboratory evidence of significant hepatocellular injury, (iii) current substance use disorders other than tobacco, (iv) current mental or psychiatric conditions or diseases that required psychoactive medication or inpatient treatment, (v) diagnosis of antisocial personality disorder, and (vi) history of psychosis. The inclusion and exclusion criteria were established according to ICD-10 criteria using a German version of the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA; Bucholz *et al.*, 1994). The severity of alcohol withdrawal was investigated with the Withdrawal Syndrome Scale (WSA; Bech *et al.*, 1989).

Blood samples for measuring free testosterone in plasma were always drawn under standardized conditions at 8.30 in the morning: on the day of admission to the clinic (Day 1), after successful completion of detoxification (Day 10), and 6 weeks later (Day 40) before discharge from the clinic. For detoxification, only clomethiazole had been used if needed. No other medication, e.g. naltrexone, was administered. Abstinence was verified by regular alcohol-breath tests.

Control subjects

The 43 members of the control group were recruited as age-matched, healthy men. Their mean age was 44.6 years (SD = 12.3). They provided data in a semi-structured psychiatric interview, to verify that none of them had any past or current history of alcoholism, drug dependency, or other psychiatric disorder or somatic disease. They were all in good health and were not taking any medication. Blood samples were taken once at 8.30 a.m. under standardized conditions.

Testosterone analysis

Blood samples were drawn into chilled tubes prepared with ethylenediamine tetraacetic acid and aprotinin, immediately spun at 4°C, and the plasma stored at -80°C. Plasma concentrations of free testosterone were determined using a commercially available radioimmunoassay (DSL-4900 ACTIVETM Free Testosterone RIA-kit, DSL Deutschland GmbH, Sinsheim, Germany). The detection limit was 0.18 pg/ml.

Statistical analysis

In order to generate subgroups for the severity of drinking and smoking, two median splits were calculated. Alcohol and tobacco use is characterized by the quantity of drinking (number of standard drinks) and smoking (number of cigarettes) during the last three months prior to admission. The median splits of quantity result in four severity states of alcohol and cigarette consumption: low alcohol–low tobacco, low alcohol–high tobacco, high alcohol–low tobacco, high alcohol–high tobacco.

All statistical analyses were performed with SPSS/12.0 for Windows. The parametric methods used were: *t*-test for independent samples for group comparisons. ANOVAs with repeated measures (Day 1, 10, and 40) and up to three group

factors (alcohol consumption, cigarette smoking, withdrawal score) were used to detect the testosterone course and the influences on it. Pearson product momentum correlations (controlled for age and weight) were calculated to evaluate the associations between testosterone concentrations and pre-treatment alcohol and tobacco use. A probability of $P < 0.05$ was defined as the level of significance.

RESULTS

Table 1 summarizes the demographic and social characteristics of the alcohol-dependent men. Pre-treatment alcohol use and tobacco use parameters as displayed in Table 2 indicate that the sample exhibited long-lasting and severe alcohol misuse.

As shown in Fig. 1, testosterone plasma levels of alcoholics on Day 1 and 10 did not differ from those of healthy controls. However, there was a significant increase in the testosterone plasma concentration until Day 40 in alcoholic men ($t = -3.15$, $df = 50$, $P < 0.001$).

The concentrations of testosterone on Day 1, 10, and 40 correlated significantly with each other ($P < 0.001$). Two significant correlations between pre-treatment alcohol parameters and testosterone were found: between total number of drinks

Table 1. Demographic and social data of the alcoholics

Demographic and social characteristics ($n = 51$)	
Age (years)	
Mean	42.8
SD	10.2
Range	22–68
Marital status; n (%)	
Married	24 (47)
Single	08 (16)
Divorced/separated	19 (37)
Living situation; n (%)	
Living alone	17 (33)
Living with a friend or relative	12 (24)
Living with the spouse	22 (43)
Highest grade in school; n (%)	
Without graduation	01 (02)
Elementary school	35 (69)
Junior high school	11 (21)
High school	01 (02)
University	03 (06)
Occupational status; n (%)	
Employed	33 (65)
Unemployed	17 (33)
Student	01 (02)

Table 2. Pre-treatment drinking and smoking characteristics of the alcoholics

Drinking and smoking characteristics ($n = 51$)	Mean	SD
Age of alcoholism onset (ICD-10) (years)	29.4	10.7
Duration of alcohol dependence (years)	12.0	8.7
Alcohol consumption during the 3 months prior to admission		
Drinking days/month	28.2	5.0
Total number of drinks	2039.6	1149.3
Nicotine use during 3 months prior to admission		
Smoking days/month	24.1	11.6
Total number of cigarettes	1768.9	1323.9

Drinks = standard drinks = 10 g ethanol

and testosterone on Day 1 ($r = 0.37$, $P < 0.01$) as well as between drinking days per months before admission and testosterone on Day 1 ($r = 0.34$, $P < 0.05$). There were also significant positive correlations between smoking variables and testosterone concentrations on Day 1 ($r = 0.29$, $P < 0.05$), and between total number of cigarettes and testosterone concentrations on Day 10 ($r = 0.31$, $P < 0.05$).

The alcoholics with lower alcohol consumption ($n = 23$, 45%) had fewer than 1800 standard drinks. The higher alcohol consumption group ($n = 28$, 51%) had drunk between 1800 and 5400 standard drinks over the preceding 3 months. Those alcoholics, with a low cigarette consumption ($n = 20$, 39%), smoked fewer than 1800 cigarettes, whereas the high cigarette consumption group ($n = 31$, 61%) had smoked a total of

1800–4500 cigarettes during the 3 months preceding the clinical treatment.

For all four groups (low alcohol-low tobacco, low alcohol-high tobacco, high alcohol-low tobacco, high alcohol-high tobacco), there was a significant increase in testosterone over the 40 days following the withdrawal ($F = 19.35$, $P = 0.0001$; Fig. 2). This increase ran more or less parallel for all groups. However, as shown in Fig. 2, the four groups differed significantly in their testosterone level: the lower the consumption of alcohol and/or cigarettes had been, the lower were the measured free testosterone concentrations during and after the withdrawal period. The severity of the withdrawal symptoms had no effect, neither on the level of testosterone nor on its course over the 40 days after withdrawal.

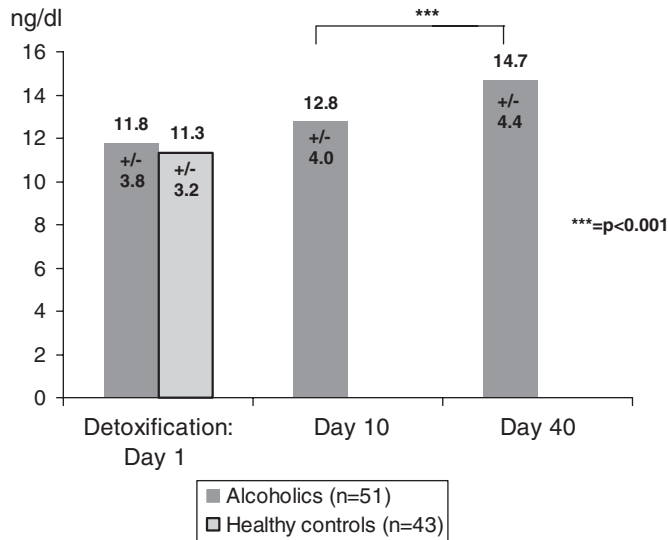


Fig. 1. Free testosterone in plasma on Day 1, 10, and 40 after detoxification in alcoholics and healthy control group.

DISCUSSION

To our knowledge, this is the first study investigating the course of plasma testosterone levels in alcohol dependent men over such a long period. Additionally, there has been no study yet investigating the combined effects of alcohol and smoking on this hormone in alcoholics.

First, our results confirm the findings of Gumus *et al.* (1998), who reported an increase in testosterone levels after alcohol cessation. However, we did not observe recovery during clinical withdrawal, but only during the following 40 days of abstinence. This indicates that, even in heavy drinking alcoholics, delayed but significant recovery in testosterone secretion is possible, as long as there is no severe liver dysfunction.

Second, we found a positive and significant association between tobacco smoking and testosterone levels at Day 1 and at Day 10. This is in accordance with the results from a population-based study reported by Svartberg *et al.* (2003). Nevertheless, our study is the first to confirm this relationship in alcoholics.

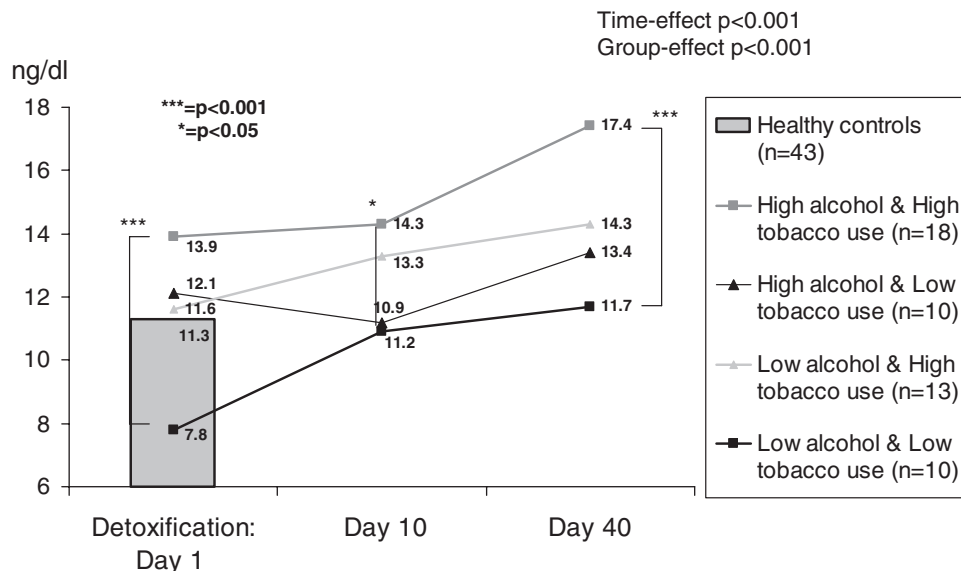


Fig. 2. Free testosterone in plasma on Day 1, 10, and 40 after detoxification in subgroups of alcoholics with high and low pre-treatment alcohol use and tobacco use.

Third, our results do not support the findings reported by Ruusa and Bergman (1996). While they found that male alcoholics with low levels of testosterone developed more symptoms during withdrawal, we could not confirm such an association. In our sample, plasma testosterone levels measured immediately before detoxification did not affect the severity of alcohol withdrawal. However, we used a specific withdrawal syndrome scale (WSA), while Ruusa and Bergman (1996) employed a comprehensive psychopathological rating scale (CPRS). Additionally, while they determined testosterone levels after a mean period of sobriety of 2.5 days, our hormone levels were always measured at Day 1 of treatment. Therefore, methodological diversities might explain, at least in part—the difference.

Finally, the main goal of this investigation was to analyse the combined effects of alcohol and smoking. For this reason, we subdivided our alcohol-dependent participants according to their (high or low) level of consumption, thus creating four subgroups: low alcohol–low tobacco (Group 1), low alcohol–high tobacco (Group 2), high alcohol–low tobacco (Group 3), and high alcohol–high tobacco (Group 4). As expected, we found higher testosterone levels in those with high tobacco consumption (Groups 2 and 4), which is in accordance with the literature reported. However, highest testosterone levels were observed in the subgroup of those with both high alcohol and high tobacco consumption (Group 4). Compared with its counter-group with low alcohol and low tobacco consumption (Group 1), the difference in testosterone levels was statistically significant at all three time-points. Since there is no precedent in the literature, we can only speculate about this unexpected phenomenon. We hypothesize that in our sample the rebound effect of testosterone recovery was highest in those who had stressed their testosterone system most, that is, in the subgroup with highest alcohol consumption before detoxification. Whatever the explanation of this phenomenon, our results demonstrate that smoking matters to testosterone levels in alcoholics. Therefore, in future studies on this topic, tobacco smoking should be considered as an important variable.

The results presented must be interpreted from the perspective of the methodologies used. One important caveat is the fact that there were only 10–18 men in the subgroups analysed. Hence, additional studies will be necessary before more definitive conclusions can be drawn.

We also measured free testosterone (not total testosterone) and sex hormone binding globulin (SHBG), which means that our perspective of testosterone is incomplete. However, Vermeulen *et al.* (1999) reported that free (i.e. bioavailable) testosterone reflects the clinical situation more precisely than the total hormone level or SHBG.

We did not control for other potentially confounding variables such as body-mass index (BMI), coffee consumption, or physical activity. Although coffee consumption and physical activity were not associated with free testosterone in a population based sample (Svartberg *et al.*, 2003) this might not necessarily apply to smoking alcoholics. Unfortunately neither the number of smokers nor the amount of tobacco used by the healthy controls was assessed.

This study sought to shed light on the complex relationship between alcohol/tobacco consumption and plasma testosterone levels in alcohol dependent men. The results support the

hypothesis that, in heavily smoking and drinking men, there is a relationship between higher levels of testosterone and higher levels of alcohol and tobacco consumption.

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REFERENCES

- Adams, M. L., Meyer, E. R. and Cicero, T. J. (1997) Interactions between alcohol- and opioid-induced suppression of rat testicular steroidogenesis *in vivo*. *Alcoholism: Clinical and Experimental Research* **21**, 684–690.
- Adler, R. A. (1992) Clinically important effects of alcohol on endocrine function. *Journal of Clinical Endocrinology and Metabolism* **74**, 957–960.
- Bech, P., Rasmussen, S., Dahl, A. *et al.* (1989) The Withdrawal Syndrome Scale for alcohol and related drugs. *Nordisk Psykiatrisk Tidsskrift* **43**, 291–294.
- Bucholz, K. K., Cadoret, R., Cloninger, C. R. *et al.* (1994) A new, semi-structured psychiatric interview for use in genetic linkage studies: a report of the reliability of the SSAGE. *Journal of Studies on Alcohol* **55**, 149–158.
- Castilla-Garcia, A., Santolaria-Fernandez, F. J., Gonzalez-Reimers, C. E. *et al.* (1987) Alcohol-induced hypogonadism: reversal after ethanol withdrawal. *Drug and Alcohol Dependence* **20**, 255–260.
- Dhawan, K. and Sharma, A. (2002) Prevention of chronic alcohol and nicotine-induced azospermia, sterility and decreased libido, by a novel tri-substituted benzoflavone moiety from *Passiflora incarnata* Linnaeus in healthy male rats. *Life Sciences* **71**, 3059–3069.
- Funabashi, T., Sano, A., Mitsushima, D. *et al.* (2005) Nicotine inhibits pulsatile luteinizing hormone secretion in human males but not in human females, and tolerance to this nicotine effect is lost within one week of quitting smoking. *Journal of Clinical Endocrinology and Metabolism* **90**, 3908–3913.
- Gumus, B., Yigitoglu, M. R., Lekili, M. *et al.* (1998) Effect of long-term alcohol abuse on male sexual function and serum gonadal hormone levels. *International Urology and Nephrology* **30**, 755–759.
- Heinz, A., Rommelspacher, H., Gräf, K.-J. *et al.* (1995) Hypothalamic-pituitary-gonadal axis, prolactin, and cortisol in alcoholics during withdrawal and after three weeks of abstinence: comparison with healthy control subjects. *Psychiatry Research* **56**, 81–95.
- Hiney, J. K. and Dees, W. L. (1991) Ethanol inhibits luteinizing hormone-releasing hormone release from the median eminence of prepubertal female rats *in vitro*: investigation of its actions on norepinephrine and prostaglandin-E₂. *Endocrinology* **128**, 1404–1408.
- Meyer, R. E. and Dolinsky, Z. S. (1990) Ethanol beverage anticipation: effects on plasma testosterone and luteinizing hormone levels—a pilot study. *Journal of Studies on Alcohol* **51**, 350–355.
- Muller, M., den Tonkelaar, I., Thijssen, J. H. *et al.* (2003) Endogenous sex hormones in men aged 40–80 years. *European Journal of Endocrinology* **149**, 583–589.
- Orpana, A. K., Harkonen, M. and Erikson, C. J. P. (1990) Ethanol-induced inhibition of testosterone biosynthesis in rat Leydig cells: role of mitochondrial substrate shuttles and citrate. *Alcohol and Alcoholism* **25**, 499–507.
- Patterson, T. R., Stringham, J. D. and Meikle, A. W. (1990) Nicotine and cotinine inhibit steroidogenesis in mouse Leydig cells. *Life Sciences* **46**, 265–272.
- Ruusa, J. and Bergman, B. (1996) Sex hormones and alcohol withdrawal: does a good supply of testosterone prevent serious symptoms during detoxification. *Alcohol* **13**, 139–145.
- Ruusa, J., Bergman, B. and Sundell, M.L. (1997) Sex hormones during alcohol withdrawal: a longitudinal study of 29 male

- alcoholics during detoxification. *Alcohol and Alcoholism* **32**, 591–597.
- Sarasin, A., Schlumpf, M., Müller, M. *et al.* (2003) Adrenal-mediated rather than direct effects of nicotine as a basis of altered sex steroids synthesis in fetal and neonatal rat. *Reproductive Toxicology* **17**, 153–162.
- Schiavi, R. C., Stimmel, B. B., Mandeli, J. *et al.* (1995) Chronic alcoholism and male sexual function. *American Journal of Psychiatry* **152**, 1045–1051.
- Svartberg, J., Midtby, M., Bonna, K. H. *et al.* (2003). The association of age, lifestyle factors and chronic disease with testosterone in men: the Tromsø Study. *European Journal of Endocrinology* **149**, 145–152.
- Tamimi, R., Mucci, L. A., Spanos, E. *et al.* (2001) Testosterone and oestradiol in relation to tobacco smoking, body mass index, energy consumption and nutrient intake among adult men. *European Journal of Cancer Prevention* **10**, 275–280.
- Uddin, S., Emanuele, M. A., Emanuele, N. V. *et al.* (1994) The effect of *in vitro* ethanol exposure on luteinizing hormone and follicle stimulating hormone mRNA levels, content, and secretion. *Endocrine Research* **20**, 201–217.
- Välimäki, M., Tuominen, J. A., Huhtaniemi, I. *et al.* (1990) The pulsatile secretion of gonadotropins and growth hormone, and the biological activity of luteinizing hormone in men acutely intoxicated with ethanol. *Alcoholism: Clinical and Experimental Research* **14**, 928–931.
- Vermeulen, A., Verdonck, L. and Kaufman, J. M. (1999) A critical evaluation of simple methods for the estimation of free testosterone in serum. *Journal of Clinical Endocrinology and Metabolism* **84**, 3666–3672.
- Walter, M., Gerhard, U., Gerlach, M. *et al.* (2006) Cortisol concentrations, stress-coping styles after withdrawal, and long-term abstinence in alcohol dependence. *Addiction Biology* **11**, 157–162.