Received: 9 August 2021 | Revised: 8 December 2021 | Accepted: 10 December 2021

DOI: 10.1111/adb.13135

ORIGINAL ARTICLE

Addiction Biology SSA WILEY

Sulphated dehydroepiandrosterone serum levels are reduced in women with alcohol use disorder and correlate negatively with craving: A sex-separated cross-sectional and longitudinal study

Christian Weinland[1] | Christiane Mühle[1] | Claudia von Zimmermann[1] | Johannes Kornhuber[1] | Bernd Lenz[1,2]

[1]Department of Psychiatry and Psychotherapy, Friedrich-Alexander University Erlangen–Nürnberg (FAU), Erlangen, Germany

[2]Department of Addictive Behavior and Addiction Medicine, Central Institute of Mental Health (CIMH), Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

Correspondence
Christian Weinland, Department of Psychiatry and Psychotherapy, Friedrich-Alexander University Erlangen–Nürnberg (FAU), Schwabachanlage 6, D-91054 Erlangen, Germany.
Email: christian.weinland@uk-erlangen.de

Funding information
Deutsche Forschungsgemeinschaft; University Hospital of the Friedrich-Alexander University Erlangen–Nürnberg (FAU)

Abstract

Previous studies have established a role of sex hormones in alcohol use disorder (AUD).Only few clinical investigations with low numbers of patients with AUD have focused on the sulphated form of dehydroepiandrosterone (DHEA-S), despite its function as a neuromodulating sex steroid on receptors in the central nervous system (γ-aminobutyric acid type A, *N*-methyl-D-aspartate, sigma-1 receptors). DHEA-S serum levels were compared between 200 inpatients with AUD (44% women) admitted for withdrawal treatment and 240 healthy controls (45% women) and analysed longitudinally in patients from early abstinence (baseline) to a median of 5 days later. We also correlated DHEA-S levels with craving, liver enzyme activities, and prospective alcohol-related readmissions during a 24-month follow-up. DHEA-S concentrations were lower in female patients than in female healthy controls during baseline (70%) and decreased from baseline to follow-up in the female and male patients groups (down to: women, 92%; men, 76%). Baseline DHEA-S concentrations correlated with the total and obsessive subscales of the Obsessive–Compulsive Drinking Scale and with maximum visual analogue scale craving scores in female patients (Rho ≤ −0.240) and gamma-glutamyl transferase activity in female (Rho = −0.292) and male (Rho = −0.391) patients. DHEA-S did not significantly predict outcome. We found interactions with smoking behaviour and age. This is the first study based on large cohorts of inpatients with AUD undergoing a qualified detoxification treatment to provide sex-separated evidence for associations of DHEA-S serum concentrations with AUD and related phenotypes. The results stimulate further investigations whether DHEA-S directly influences alcohol craving building a basis to develop sex-sensitive prevention and treatment strategies.

KEYWORDS
AUD, craving, DHEA -S, relapse, sex hormones

1 | INTRODUCTION

Research on the role of sex steroids in alcohol use disorder (AUD) has contributed to a better understanding of neurobiological mechanisms underlying both AUD per se and sex differences in AUD.[1–5] Studies on sex hormone activity in AUD addressed long-term (i.e., organizational) and short-term (i.e., functional) effects of primarily androgens such as testosterone or dihydrotestosterone[3,6–9] and to a lesser extent oestrogen and progesterone.[10,11] Despite their central function as precursors of sex hormones,[12] there are only few studies which have investigated how dehydroepiandrosterone (DHEA) and its sulphated form dehydroepiandrosterone sulphate (DHEA-S) are associated with AUD. Both are involved in neurobiological processes and are therefore considered to act as neural modulators.[12–15] The production of DHEA and its sulphation via the enzyme hydroxysteroid sulphotransferase to DHEA-S is primarily located in the *zona reticularis* of the adrenal cortex.[16] There is evidence from animal research on amphibians that DHEA-S is also formed in the central nervous system.[17] However, studies on mammalians failed to locate the enzyme hydroxysteroid sulphotransferase in the brain.[18] While DHEA serves as a precursor hormone in the biosynthesis of androgen and oestrogen sex steroids and plays a fundamental physiological role in steroid metabolism,[19] the sulphated form is primarily active as a neurosteroid.[20–22] Thus, DHEA-S has been described as an allosteric modulator of *N*-methyl-D-aspartate (NMDA) receptor,[23,24] the γ-aminobutyric acid type A (GABA-A),[21] and the sigma-1 receptors.[25] The activation of these receptors is linked to non-genomic and therefore direct and short-term mechanisms.[12]

Animal studies give preclinical insight on the cerebral interaction of DHEA-S with ethanol in vertebrates. Experiments comparing alcohol-dependent with non-alcohol-dependent rats show that the administration of DHEA-S influences the effects of GABA-A receptor-binding substances.[26] In male mice, the treatment with DHEA-S promotes an accelerated tolerance to the effects of alcohol on motoric impairment via the GABA-A and NMDA receptor systems.[27] Also, DHEA-S administration to mice exerts anxiolytic effects that, however, are dose-dependent and block the anxiolytic effects of alcohol in the higher dose range.[28] Moreover, Sharma et al.[29] demonstrated in alcohol-withdrawing rats attenuated withdrawal-related fears after administration of DHEA-S.

Numerous clinical association studies have investigated a possible influence of acute and long-term alcohol consumption on serum DHEA-S levels in non-dependent individuals. In summary, and despite relevant differences of the cohorts and study designs (i.e., sex, age, acute vs. chronic alcohol consumption, premenopausal and postmenopausal women, and cross-sectional and longitudinal studies) the majority of these studies found higher DHEA-S serum levels to associate with alcohol consumption in both women and men.[30–36] These relationships have also been reported in saliva samples.[37] However, some studies failed to show an association[38,39] or found lower levels in alcohol consuming subjects.[40]

DHEA-S serum levels were also analysed in cohorts of patients diagnosed with AUD. The investigation of DHEA-S serum levels was overall carried out in relatively small study cohorts. The studies focused on the course of DHEA-S serum levels over different periods of abstinence. Some of the patients investigated were already abstinent or were receiving a withdrawal treatment. Moreover, the studies were primarily carried out in either men or women.

Differences in DHEA-S serum concentrations between patients with AUD and healthy controls were found in two studies. Välimäki et al.[41] examined 12 alcohol-dependent premenopausal women during withdrawal treatment and 11 healthy premenopausal women and analysed DHEA-S serum concentrations from blood samples drawn in various phases of the menstrual cycle. Females with AUD exhibited significantly lower DHEA-S concentrations than control subjects only in the follicular phase of the cycle. In a further study with 30 alcohol-dependent men, basal serum DHEA-S concentrations were determined during withdrawal treatment on days 1, 8, and 28. The data were compared with 20 healthy controls and analysed over the time course. The group comparisons showed significantly lower concentrations in patients on days 8 and 28 of withdrawal without differences on day 1. The longitudinal analyses revealed significantly reduced DHEA-S concentrations on day 8 of withdrawal. Subsequently, the patients were separated dichotomously in two groups showing either tendency to more or less aggressive behaviour in their biography to secondarily perform group comparisons and longitudinal analyses. In group comparisons (healthy controls vs. patients), significantly lower DHEA-S serum concentrations were measured in the low aggressive subcohort on days 8 and 28, while in the aggressive subcohort, serum DHEA-S levels differed significantly with lower levels on days 1 and 8 of withdrawal, but not on day 28. Longitudinal analyses revealed significant differences only in the low-aggression cohort.[42]

However, also null results have been reported. In a study conducted by Adinoff et al.,[43] different groups of patients (*n*: 10, 10, 11) were compared during different durations of abstinence (weeks 1 and 2 of abstinence, abstinence between the 3rd week and 6 months, and abstinence over 6 months) and with 19 healthy controls. In this study, the DHEA-S concentrations showed no significant differences between the respective intervals of abstinence and in the comparison with healthy controls. Another study on 15 male and 6 female patients, who had been abstinent for already 4 weeks, and healthy controls (7 men/4 women) did also not reveal any significant differences in the DHEA-S serum concentrations.[44] Hill et al.[45] determined the concentration of DHEA-S in 20 premenopausal females with AUD during withdrawal treatment and compared these over the course of abstinence during 3 and 14 days and 1 and 4 months, respectively, after treatment and also in comparison with controls. Again, neither the longitudinal analyses of the concentrations nor the group comparison showed any significant differences in the DHEA-S serum concentrations.

The previous studies indicate that DHEA-S serum concentrations are reduced in AUD, although smaller studies were not able to show this effect, presumably due to insufficient power. However, we lack clinical studies that have examined DHEA-S in the serum in cross-sectional and longitudinal designs with larger cohorts of patients and

healthy controls and a balanced distribution of women and men. There is also need for an investigation analysing associations of DHEA-S concentrations with phenotypes of AUD, that is, the extent of craving during withdrawal, the liver enzyme activities as clinical routine parameters for chronic alcohol consumption, and outcome parameters post-inpatient treatment course.

1.1 | Aims of the study

As far as we know, this is the first study to sex-separately investigate associations of serum DHEA-S concentrations with AUD using a cross-sectional and longitudinal study design with a large and sex-balanced study population. We tested whether DHEA-S serum levels differ between patients with AUD and healthy control subjects, analysed the effect of withdrawal treatment and abstinence in early states on the serum concentrations, and investigated correlations with craving for alcohol, liver enzyme activities as an indirect marker of alcohol use, and prospective alcohol-related readmissions over a 24-month period. We also explored associations of DHEA-S serum levels with sex and age. Because tobacco use is a highly prevalent comorbidity in individuals with AUD and because of evidence that smoking interacts with DHEA-S in serum,[46] we also analysed possible interactions with the parameters of interest in this study.

2 | MATERIALS AND METHODS

2.1 | Study cohort

The collected data refer to a prospective, cross-sectional, and bicentric study cohort enrolled between 2013 and 2014 to investigate mechanisms and biomarkers of AUD and its phenotypes (Neurobiology of Alcoholism [NOAH]).[3,10,11,47–57] For this project, 200 inpatients with AUD (n [women] = 87, n [men] = 113) and 240 healthy control subjects (n [women] = 107, n [men] = 133) were recruited and matched according to age and sex. The patients fulfilled the diagnostic criteria of AUD according to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) and alcohol dependence according to the tenth revision of the International Classification of Diseases (ICD-10). We included 171 out of 903 screened inpatients at the *Klinik für Psychiatrie, Sucht, Psychotherapie und Psychosomatik of the Klinikum am Europakanal* and 29 out of 85 screened inpatients at the University Hospital Erlangen Department of Psychiatry and Psychotherapy. Both centres are located in the German Franconian city of Erlangen and are specialized treatment centres for mental health. All patients were admitted for inpatient withdrawal treatment. Predefined exclusion criteria were refusal to sign an informed consent (n = 379); other psychiatric comorbidities, including schizophrenia, depression with affective symptoms independent from alcohol use, or substance use disorder other than alcohol or nicotine (n = 194); severe somatic disorders (n = 90); abstinence longer than 72 h prior to study inclusion (n = 88); non-Caucasian ethnicity (n = 3); other reasons (n = 34). The predefined inclusion criteria included age at least of 18 years and early-abstinence (last alcohol intake within the preceding 24–72 h).

The control subjects were recruited via flyers and online advertising. In telephone interviews, a total of 1215 control subjects were screened, checked for eligibility, excluded in case of prior inpatient mental health care during their life time or any psychotherapeutic or psychiatric outpatient treatment during the past 10 years.

2.2 | Sociodemographic characteristics, medical data, blood samples, and assessment of craving

All patients were visited twice; the first visit (baseline, T0) was scheduled during early abstinence, the second visit at median 5 days later [T1 (interquartile range [IQR] 3/6; participation rate of 81.5% of the patients)]. Healthy control subjects attended a single study visit. Sociodemographic characteristics and medical data were gathered during the first study visit. In the patients, blood samples were drawn at both study visits; craving was quantified at T0 using the German version of the well-established Obsessive–Compulsive Drinking Scale (OCDS[58,59]) and, at T1, we used visual analogue scales (VAS) to assess average, maximum, and frequency of craving during the previous 7 days period prior to the second study visit.

2.3 | Measurement of serum DHEA-S levels and liver enzyme activities

To minimize circadian effects, all blood samples were taken in the morning (7:30 AM–11:00 AM). Aliquots of serum centrifuged at 2000 × g for 10 min were transferred to −80°C for storage until use. Serum DHEA-S was quantified in duplicates of 50 μl using the competitive ELISA RE52181 (intra-assay coefficient of variation [cv] 2%, inter-assay cv 2%, IBL, Germany).

Carbohydrate-deficient transferrin (CDT), glutamic oxaloacetic transaminase (GOT), glutamate-pyruvate transaminase (GPT), and gamma-glutamyl transferase (GGT) were determined by the Central Laboratory of the *Universitätsklinikum Erlangen*, Germany (DIN EN ISO 15189 accredited), from separately collected serum vials.

2.4 | Prospective outcome measures

The outcome was evaluated during a 24-month observation period using the following surrogate parameters for relapse: alcohol-related hospital readmission yes versus no, the total number of readmissions during the observation period, and the number of days to the first readmission. The data were extracted from the electronic patient records at the two study centres. In patients without any alcohol-related readmission during the observation window, 'days to first readmission' were set to 730 for statistical analyses.

2.5 | Statistical analyses

The data were analysed with SPSS for Windows 27.0 (SPSS Inc., Chicago, IL, USA). The descriptive statistics report frequencies, medians and IQRs as calculated by the custom tables function. The Spearman method was employed to calculate bivariate correlations, the Mann–Whitney *U* and Wilcoxon tests to calculate cross-sectional and longitudinal differences because some variables deviated significantly from normal distribution according to the Kolmogorov–Smirnov test. The χ^2 test was employed in order to evaluate differences in the frequency of nominal variables.

We here tested 30 main DHEA-S hypotheses and used the Bonferroni method to estimate type 1 error risk (15 hypotheses for each sex): 3 cross-sectional tests (patients T0 vs. controls, patients T1 vs. controls, patients with readmission vs. patients without readmission), 1 longitudinal test (patients T0 vs. patients T1), 11 correlations with OCDS-total, OCDS-obsessive, and OCDS-compulsive during T0, average, maximum, and frequency of VAS craving during the 7 days before T1, GOT, GPT, and GGT activity during T0, and latency to the first alcohol-related readmission and total number of readmissions during the 24-month observation period. We evaluated women and men separately because of the sex differences in AUD[1,4] and the need for sex-specific analysis to improve science in general.[60]

2.6 | Ethics statement

Written informed consent to the participation was obtained from all study subjects. The study was approved by the Ethics Committee of the Medical Faculty of the Friedrich-Alexander University Erlangen–Nürnberg (ID 81_12 B).

3 | RESULTS

3.1 | Sociodemographic characteristics

In separated analyses for women and men, the patients with AUD did not significantly differ from the healthy control subjects in terms of age, fasting status, and postmenopausal status. However, we found significantly fewer months of employment during the previous year (women, 63%; men, 13%), fewer years at school (women, 92%; men, 83%), and higher CDT levels (women, 132%; men, 194%) in the patients with AUD than in the healthy control subjects. The patients with AUD were also significantly more likely to be smokers than the controls (odds ratio [OR]; women, 14.5; men, 12.6; Table 1).

3.2 | DHEA-S serum levels: Cross-sectional and longitudinal differences in AUD

In female patients with AUD, the DHEA-S levels were significantly lower than in female healthy controls (70%) and decreased significantly from baseline during early abstinence to the follow-up at median 5 days following the baseline (down to 92%) (Figure 1A, Table S1). There were no significant cross-sectional differences between male patients with AUD and male healthy controls, but a significant reduction between baseline and 5-day follow-up (down to 76%) in male patients with AUD (Figure 1B, Table S1). The decline in DHEA-S concentrations from baseline to the follow-up in female and male patients with AUD and the lower DHEA-S concentrations in female patients with AUD during the follow-up relative to the female healthy controls remained significant after Bonferroni adjustment.

3.3 | DHEA-S serum levels: Correlations with alcohol craving and liver enzyme activities in patients with AUD

In female patients with AUD, higher baseline DHEA-S levels correlated significantly with lower craving (Table S2), that is, lower OCDS-total scores (Rho = −0.273; Figure 1C), lower OCDS-obsessive scores (Rho = −0.240; Figure 1D), and lower maximum VAS craving scores (Rho = −0.357; Figure 1E). At baseline, higher DHEA-S levels correlated also significantly with lower liver enzyme activities in both sexes (Table S2; female patients with AUD: GGT, Rho = −0.292, Figure 1F; male patients with AUD: GOT, Rho = −0.218, Figure 1G, GGT, Rho = −0.391, Figure 1H). The negative correlation between DHEA-S concentration and GGT activity in male patients with AUD remained significant after Bonferroni adjustment.

3.4 | DHEA-S serum levels: Prediction of alcohol-related readmissions

Neither in male nor in female patients with AUD, baseline DHEA-S levels predicted significantly the risk of, the number of, or the latency to alcohol-related readmissions (Table S3).

3.5 | DHEA-S serum levels: Associations with sex, age, and smoking status

The DHEA-S levels were consistently lower in women than in men (Table S1; patients with AUD, baseline, 48%, $U = 2758$, $p < 0.001$, 58%, follow-up, $U = 1715$, $p < 0.001$; healthy control subjects, 77%, $U = 4962$, $p < 0.001$). In sex- and group-separated analyses, DHEA-S levels correlated significantly and consistently with age (female patients with AUD: $N = 87$, Rho = −0.274, $p = 0.010$; male patients with AUD: $N = 113$, Rho = −0.255, $p = 0.006$; female healthy controls: $N = 107$, Rho = −0.526, $p < 0.001$; male healthy controls: $N = 133$, Rho = −0.618, $p < 0.001$). Moreover, DHEA-S levels were not significantly related to smoking status except for higher levels in smoking than non-smoking female patients with AUD (185%) (Figure 1I, Table S4), and this difference might be explained by a younger age of smoking than non-smoking female patients with AUD ($U = 353$, $p = 0.026$).

TABLE 1 Sociodemographic characteristics

	Patients with AUD				Healthy control subjects				AUD vs. control group	
	N	M/F	IQR		*N*	M/F	IQR		*U* or χ^2	*p*
Women										
Age (years)	87	48	42	55	107	49	39	55	4542	0.772[a]
Months of employment (previous year)	70	7.5	0.0	12.0	107	12.0	5.0	12.0	3014	**0.013**[a]
Years at school	74	12.0	10.0	14.0	107	13.0	12.0	15.0	2714	**< 0.001**[a]
Fasting (%)	80	18	—	—	101	26	—	—	1.8	0.184[b]
Postmenopausal status (%)	73	51	—	—	100	44	—	—	0.8	0.384[b]
Alcohol concentration at admission (‰)	85	1.2	0.1	1.8	—	—	—	—	—	—
Previous withdrawal treatments (n)	58	5	2	11	—	—	—	—	—	—
CDT (nephelometry, %)	87	1.9	1.6	2.5	107	1.5	1.3	1.6	1415	**< 0.001**[a]
AUDIT score	—	—	—	—	96	3	2	4	—	—
Smokers (%)	78	77	—	—	107	19	—	—	62.3	**< 0.001** [b]
24-month alcohol-related readmissions										
Readmission rate (%)	87	52.9	—	—	—	—	—	—	—	—
Total number	87	1	0	3	—	—	—	—	—	—
Latency (days)	87	625	90	≥ 730	—	—	—	—	—	—
Men										
Age (years)	113	48	40	53	133	48	38	56	7369	0.794[a]
Months of employment (previous year)	96	1.5	0.0	12.0	131	12.0	3.0	12.0	3994	**< 0.001**[a]
Years at school	97	12.0	10.5	15.0	132	14.5	13.0	17.0	4273	**< 0.001**[a]
Fasting (%)	103	16	—	—	127	24	—	—	2.8	0.097[b]
Alcohol concentration at admission (‰)	108	1.7	0.5	2.4	—	—	—	—	—	—
Previous withdrawal treatments (n)	89	6	2	12	—	—	—	—	—	—
CDT (nephelometry, %)	113	2.8	1.9	4.0	132	1.5	1.3	1.7	1636	**< 0.001**[a]
AUDIT score	—	—	—	—	125	4	3	6	—	—
Smokers (%)	104	78	—	—	133	22	—	—	73.8	**< 0.001**[b]
24-month alcohol-related readmissions										
Readmission rate (%)	113	67.3	—	—	—	—	—	—	—	—
Total number	113	2	0	4	—	—	—	—	—	—
Latency (days)	113	285	57	≥ 730	—	—	—	—	—	—

Note: $p < 0.05$ in bold.
Abbreviations: AUD, alcohol use disorder; AUDIT, Alcohol Use Disorders Identification Test; CDT, carbohydrate-deficient transferrin.
The table shows the valid number of subjects analysed (*N*), medians (M) or relative frequencies (F), interquartile ranges (IQRs) and the results of [a]Mann–Whitney *U* and [b]$\chi 2$ tests.

As we found evidence for associations of DHEA-S concentrations with age and smoking status in our cohort, we explored whether these parameter might also account for the above reported results concerning the associations of DHEA-S levels with AUD.

3.6 | Influence of age and smoking status on cross-sectional and longitudinal differences

The sex-separated samples did not significantly differ with regard to age, which is thus unlikely to play a role in the here reported differences. Due to the significant association between DHEA-S and smoking status in female patients with AUD, we separated the group for smoking status and reanalysed the data. In line with the findings in the total female group, we observed DHEA-S levels to significantly decrease from baseline to follow-up in smoking female patients with AUD (down to 87%) and to be lower in non-smoking female patients than in non-smoking female healthy control subjects (baseline: 47%; follow-up: 39%) (Table S5).

3.7 | Influence of age and smoking status on correlations with alcohol craving and liver enzyme activities

Age did not significantly correlate with the craving measures in female patients with AUD or with liver enzyme activities in female or male

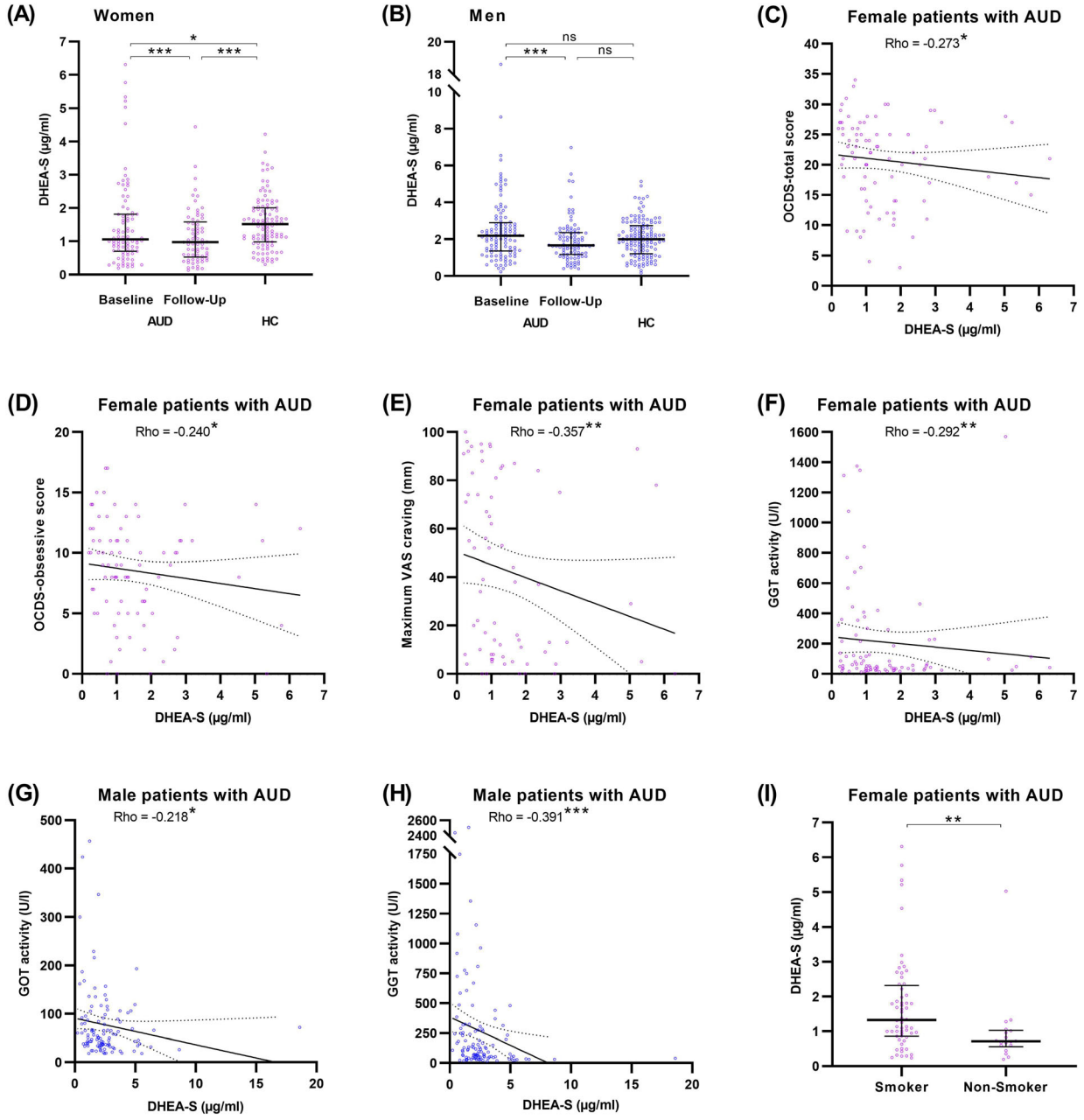

FIGURE 1 Cross-sectional and longitudinal associations between sulphated dehydroepiandrosterone (DHEA-S) serum levels, craving, liver enzyme activity, and smoking behaviour in female and male patients with alcohol use disorder (AUD) and healthy control subjects. The DHEA-S levels were significantly lower in female patients with AUD than in female control subjects (both at baseline and at follow-up) and decreased significantly from baseline to follow-up in female and male patients with AUD (A, B). The baseline DHEA-S levels correlated with Obsessive–Compulsive Drinking Scale (OCDS)-total ($Y = -0.6433 * X + 21.73$) (C), OCDS-obsessive ($Y = -0.4220 * X + 9.170$) (D), and maximum visual analogue scale (VAS) cravings ($Y = -5.335 * X + 50.44$) (E), and GGT activity ($Y = -22.35*X + 244.5$) (F) in female patients with AUD and with GOT activity ($Y = -5.457*X + 90.76$) (G) and GGT activity ($Y = -47.18*X + 383.9$) (H) in male patients with AUD. In female patients with AUD, the baseline DHEA-S levels were higher in smokers than in non-smokers (I). The graphs present medians with interquartile range (A, B, I) or Rho from Spearman correlations and best-fit lines from regression analysis with 95% confidence intervals (C–H). *p* values from Mann–Whitney *U* tests or Spearman correlations. GOT, glutamic-oxaloacetic transaminase; GGT, gamma-glutamyl transferase; ns, not significant. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

patients with AUD (Table S6); it is thus unlikely that age accounts for the observed associations of DHEA-S levels with craving or liver enzyme activities in our female sample. We found significantly lower maximum VAS craving (97%) and GOT (55%), GPT (48%), and GGT (16%) activities in female smoking than non-smoking patients with AUD (Table S7). Due to the significant association between DHEA-S and smoking status in female patients with AUD reported above, we again separated the group by smoking status and reanalysed the data.

In the female patient groups separated according to smoking status, DHEA-S did not significantly correlate with craving or liver enzyme activities (Table S8). Hence, we cannot exclude that smoking behaviour accounts for the associations between DHEA-S, craving and liver enzyme activities observed in the total group of female patients with AUD.

4 | DISCUSSION

As far as we know, the present study is the first to investigate DHEA-S serum concentrations in a large and sex-balanced cohort of patients with AUD and healthy control subjects and shows (i) lower DHEA-S concentrations in female patients relative to female healthy controls in cross-sectional analyses, (ii) longitudinal decreases of DHEA-S during inpatient withdrawal treatment and (iii) correlations of DHEA-S with the extent of craving and serum activities of liver enzymes in both female and male patients with AUD. We here conducted sex-separated analyses to provide evidence for both women and men, which is an important basis to develop sex-sensitive strategies. Furthermore, we systematically analysed the influence of smoking behaviour on the observed associations.

4.1 | Female patients with AUD: Cross-sectional and longitudinal analyses

We found lower DHEA-S serum concentrations in female patients with AUD than in female healthy control subjects. Previous data from investigations on women with AUD have shown inconsistent findings in group comparisons, mostly without significant differences.[41,44,45,61] However, the cohorts and the design of these studies differed from the present investigation in important aspects. This includes lower numbers of subjects enrolled. In some cases, blood samples of controls were compared with patients, who were abstinent for a longer period after detoxification treatment than in this study,[44,45] and in one project patients were investigated during pregnancy.[61] Välimäki et al.[41] reported lower serum levels in females with AUD only in the follicular phase of the female cycle, which might have resulted in the null findings of other studies. In our longitudinal analyses, the concentrations decreased during withdrawal in female patients. In contrast, Hill et al.[45] reported no significant changes in DHEA-S serum concentrations during withdrawal treatment and in the further course of abstinence. This inconsistency to our finding might be explained by the fact that the blood samples were drawn in other time frames than in the present study. We also found that smoking interacted with DHEA-S in female patients with AUD. Thus, we conducted subsequent analyses according to the smoking status and were able to verify a significant difference between non-smoking females with AUD and non-smoking female controls. In summary, this study's findings, which are based on a large cohort and sex-separated analyses, provide evidence that DHEA-S serum levels are lower in female patients with AUD than female healthy controls and even decrease further during withdrawal. Together with other investigations, this suggests a complex adaptation process during short- and long-term periods of withdrawal and abstinence.

4.2 | Female patients with AUD: Associations of DHEA-S serum levels with craving and liver enzyme activities

In this study, higher DHEA-S were linked to lower craving and lower GGT which might indicate a possible protective effect of DHEA-S. Smoking behaviour was also associated with DHEA-S in serum, craving and liver enzymes. Here, we were not able to verify these correlations in subgroup analyses after separation by smoking status. As the number of cases was small in these post hoc analyses, further investigations on larger cohorts with particularly non-smoking female patients are needed.

The findings concerning the effects on craving in females might be of clinical relevance. Preclinical research in mice and rats has found that DHEA-S acts as an allosteric modulator on cerebral GABA-A receptors.[26–29] Activation of the receptor via γ-aminobutyric acid (GABA) mediates anticonvulsive, sedative and anxiolytic effects,[62,63] and treatment with GABA-enhancing substances, that is, benzodiazepines and clomethiazole, attenuate withdrawal-associated symptoms such as anxiety and internal tension in patients with AUD. Sharma et al.[29] have reported that administration of DHEA-S prevents the development of tolerance against ethanol anxiolysis and withdrawing-associated anxiety in rats. Melchior and Ritzmann[28] have also shown anxiolytic effects of administered DHEA-S in male mice. Taken together, these findings are in line with the inverse correlation of craving DHEA-S levels in our clinical association study.

4.3 | Male patients with AUD: Cross-sectional and longitudinal analyses and associations with craving and liver enzyme activities

In the male group, we found no significant difference between patients with AUD at baseline and healthy controls in our cross-sectional analyses, although there was a trend for lower DHEA-S concentrations in patients during the direct follow-up relative to the healthy controls. This partly agrees with previous data in mixed cohorts and studies on males with AUD. It underlines the need for sex-separated studies and highlights the sex-separated role of sex hormones. The general higher values of DHEA-S in men than women might account for the sex-separated effect of DHEA-S concentrations in AUD. Ozsoy and Esel[42] reported lower DHEA-S levels in a cohort of 30 males with AUD during withdrawal therapy than in healthy control subjects and a decrease in concentration in the subgroup with a history of low aggression behaviour in earlier states of abstinence during withdrawal followed by an increase during the further course. As

the period between baseline and direct follow-up in this study was at median 5 days, the decline of DHEA-S levels might hence be an early effect after having stopped alcohol drinking. Once more, this underlines the importance to assume a possible effect of time or duration of abstinence on DHEA-S concentrations and highlights the need for long-term longitudinal observations and a higher frequency of study visits.

In both analyses before and after dividing male patients according to their smoking status, DHEA-S concentrations were not significantly linked to craving. These null findings certainly need replication in the future. As measurement of craving might be biased due to self-rating, a sex-dependent tendency in the rating process cannot be excluded, although we found no significant sex differences in the craving measures (data not shown). In addition, the negative correlations of the liver enzymes GOT and GGT with DHEA-S requires replication to discuss and investigate underlying causal mechanisms.

4.4 | Strengths and limitations

Compared with previous studies on this topic, we investigated large cohorts of both sexes, providing sufficient power for separated analyses in women and men and subgroups according to potential biases. Our results highlight the necessity of sex-separated addiction research and give impetus to develop sex-sensitive treatment strategies. The patients were carefully included in two specialized treatment centres in Middle Franconia, Germany. Patients with other substance use disorders besides nicotine as well as severe psychiatric comorbidities were excluded. Blood samples were drawn in a fixed time window during the first half of the day so that circadian fluctuations could be minimized. The role of tobacco smoking in our analysis highlights the importance to consider smoking as a biasing factor in research of AUD.

Taking blood samples twice during withdrawal enabled us to record fluctuations of the DHEA-S serum levels during withdrawal. However, other studies show that the decrease of DHEA-S during withdrawal might interact with time[42] and with the menstrual cycle in premenopausal females,[41] and studies with long-term observations following withdrawal failed to record significant fluctuations of serum concentrations.[44,45,61] Thus, our study cannot answer whether there are any long-term adaptation processes especially in AUD during longer periods of abstinence. This might also explain the null findings in outcome analyses following withdrawal treatment in our cohort.

Although we recruited a higher number of cases, the number of patients might not be sufficient to verify all of our hypotheses. In total, we defined 30 main DHEA-S hypotheses (8 tests on cross-sectional and longitudinal differences and 22 correlations with craving, liver enzyme activities, and outcome). Using the conservative Bonferroni approach to address the problem of multiple comparisons and type 1 error, this study still shows the significant decline in DHEA-S concentrations from baseline to follow-up in both female and male patients with AUD, the significantly lower DHEA-S concentrations in female patients with AUD during the follow-up vs. the female healthy controls, and the significant negative correlation between DHEA-S concentrations and GGT activity in male patients with AUD. The recruitment of our patients in central and specialized treatment centres was limited to the area of Middle Franconia. Thus, we cannot rule out a bias due to regional selection. As a fundamental problem of associative investigations in clinical research, it has to be questioned whether the found associations represent causality. The modulating effects of DHEA-S on cerebral receptors in animal models seem to be complex[27–29] and the transferability on cerebral processes in humans is limited. In general and beyond transferability from animal models on human cerebral biology, it should be critically taken into account, whether DHEA-S serum concentrations are linked to neurobiological actions of DHEA-S in any way. Future studies are needed to clarify this aspect and if so, whether dysregulated DHEA-S concentrations and cerebral actions are related to the addictive behaviours. It also might be possible that certain patterns of consumption, that is, the amount, frequency, and duration of heavy drinking exhibit differing effects on DHEA-S blood concentrations.

5 | CONCLUSIONS

Our study gives new sex-separated evidence for distinct associations of DHEA-S with addictive alcohol use. Furthermore, it gives reason for future investigations to provide better insight into the effects of DHEA-S on alcohol craving as well as drinking and builds a basis to develop sex-sensitive prevention and treatment strategies.

ACKNOWLEDGEMENTS

We thank Dr Andreas Ahnert, Ute Hamers and Dr Kristina Bayerlein for the opportunity and the support to recruit patients at the *Klinik für Psychiatrie, Psychotherapie, Psychosomatik* of the *Klinikum am Europakanal Erlangen*. We gratefully appreciate the support of Dr Birgit Braun, Juliane Behrens, Sarah Kubis, Dr Katrin Mikolaiczik, Sarah Saigali, Marina Sibach, and Petya Tanovska in recruiting patients and control subjects. We are thankful to Franziska Kress, Hedya Riesop, and Marcel-René Muschler for excellent technical support. We also thank for the constructive and helpful comments and suggestions during peer review. Open Access funding enabled and organized by Projekt DEAL.

FUNDING INFORMATION

This work was funded by intramural grants from the University Hospital of the Friedrich-Alexander University Erlangen–Nürnberg (FAU). It was funded in part by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)—Project-ID 402170461—TRR265.[64] CM is an associated fellow of the research training group 2162 'Neurodevelopment and Vulnerability of the Central Nervous System' funded by the DFG—270949263/GRK2162. The funders had no role in the study design, data collection, analysis, decision to publish, or preparation of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: CW, CM, BL. Performed the experiments: CW, CM, BL. Analysed the data and wrote the paper: CW, CM, BL. Commented on the manuscript and provided intellectual input: CvZ, JK. Provided resources: JK.

DATA AVAILABILITY STATEMENT

The datasets are available on request. The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ORCID

Christian Weinland https://orcid.org/0000-0002-0007-4902
Christiane Mühle https://orcid.org/0000-0001-7517-9154
Claudia von Zimmermann https://orcid.org/0000-0003-3522-754X
Johannes Kornhuber https://orcid.org/0000-0002-8096-3987
Bernd Lenz https://orcid.org/0000-0001-6086-0924

REFERENCES

1. Clayton JA, Collins FS. Policy: NIH to balance sex in cell and animal studies. *Nature*. 2014;509(7500):282-283. doi:10.1038/509282a
2. Grant BF, Goldstein RB, Saha TD, et al. Epidemiology of DSM-5 alcohol use disorder: results from the National Epidemiologic Survey on Alcohol and Related Conditions III. *JAMA Psychiat*. 2015;72(8):757-766. doi:10.1001/jamapsychiatry.2015.0584
3. Lenz B, Mühle C, Braun B, et al. Prenatal and adult androgen activities in alcohol dependence. *Acta Psychiatr Scand*. 2017;136(1):96-107. doi:10.1111/acps.12725
4. Lenz B, Müller CP, Stoessel C, et al. Sex hormone activity in alcohol addiction: integrating organizational and activational effects. *Prog Neurobiol*. 2012;96(1):136-163. doi:10.1016/j.pneurobio.2011.11.001
5. Peacock A, Leung J, Larney S, et al. Global statistics on alcohol, tobacco and illicit drug use: 2017 status report. *Addiction*. 2018;113(10):1905-1926. doi:10.1111/add.14234
6. Lenz B, Jacob C, Frieling H, et al. Polymorphism of the long polyglutamine tract in the human androgen receptor influences craving of men in alcohol withdrawal. *Psychoneuroendocrinology*. 2009;34(7):968-971. doi:10.1016/j.psyneuen.2009.01.009
7. Hermans EJ, Bos PA, Ossewaarde L, Ramsey NF, Fernández G, van Honk J. Effects of exogenous testosterone on the ventral striatal BOLD response during reward anticipation in healthy women. *Neuroimage*. 2010;52(1):277-283. doi:10.1016/j.neuroimage.2010.04.019
8. Milivojevic V, Kranzler HR, Gelernter J, Burian L, Covault J. Variation in genes encoding the neuroactive steroid synthetic enzymes 5α-reductase type 1 and 3α-reductase type 2 is associated with alcohol dependence. *Alcohol Clin Exp Res*. 2011;35(5):946-952. doi:10.1111/j.1530-0277.2010.01425.x
9. Lenz B, Schöpp E, Müller CP, Bleich S, Hillemacher T, Kornhuber J. Association of V89L SRD5A2 polymorphism with craving and serum leptin levels in male alcohol addicts. *Psychopharmacology (Berl)*. 2012;224(3):421-429. doi:10.1007/s00213-012-2770-5
10. Mühle C, Barry B, Weinland C, Kornhuber J, Lenz B. Estrogen receptor 1 gene variants and estradiol activities in alcohol dependence. *Prog Neuropsychopharmacol Biol Psychiatry*. 2019;92:301-307. doi:10.1016/j.pnpbp.2019.01.008
11. Weinland C, Mühle C, Kornhuber J, Lenz B. Progesterone serum levels correlate negatively with craving in female postmenopausal inpatients with alcohol use disorder: a sex- and menopausal status-separated study. *Prog Neuropsychopharmacol Biol Psychiatry*. 2021;110278. doi:10.1016/j.pnpbp.2021.110278
12. Prough RA, Clark BJ, Klinge CM. Novel mechanisms for DHEA action. *J Mol Endocrinol*. 2016;56(3):R139-R155. doi:10.1530/JME-16-0013
13. Baulieu EE. Neurosteroids: of the nervous system, by the nervous system, for the nervous system. *Recent Prog Horm Res*. 1997;52:1-32.
14. Lazaridis I, Charalampopoulos I, Alexaki VI, et al. Neurosteroid dehydroepiandrosterone interacts with nerve growth factor (NGF) receptors, preventing neuronal apoptosis. *PLoS Biol*. 2011;9(4):e1001051. doi:10.1371/journal.pbio.1001051
15. Pediaditakis I, Iliopoulos I, Theologidis I, et al. Dehydroepiandrosterone: an ancestral ligand of neurotrophin receptors. *Endocrinology*. 2015;156(1):16-23. doi:10.1210/en.2014-1596
16. Rainey WE, Nakamura Y. Regulation of the adrenal androgen biosynthesis. *J Steroid Biochem Mol Biol*. 2008;108(3–5):281-286. doi:10.1016/j.jsbmb.2007.09.015
17. Beaujean D, Mensah-Nyagan AG, Do-Rego JL, Luu-The V, Pelletier G, Vaudry H. Immunocytochemical localization and biological activity of hydroxysteroid sulfotransferase in the frog brain. *J Neurochem*. 1999;72(2):848-857. doi:10.1046/j.1471-4159.1999.720848.x
18. Sharp S, Barker EV, Coughtrie MW, Lowenstein PR, Hume R. Immunochemical characterisation of a dehydroepiandrosterone sulfotransferase in rats and humans. *Eur J Biochem*. 1993;211(3):539-548. doi:10.1111/j.1432-1033.1993.tb17580.x
19. Labrie F, Luu-The V, Bélanger A, et al. Is dehydroepiandrosterone a hormone? *J Endocrinol*. 2005;187(2):169-196. doi:10.1677/joe.1.06264
20. Monnet FP, Mahé V, Robel P, Baulieu EE. Neurosteroids, via sigma receptors, modulate the [3H]norepinephrine release evoked by *N*-methyl-D-aspartate in the rat hippocampus. *Proc Natl Acad Sci U S A*. 1995;92(9):3774-3778. doi:10.1073/pnas.92.9.3774
21. Svob Strac D, Jazvinscak Jembrek M, Erhardt J, Mirkovic Kos K, Pericic D. Modulation of recombinant GABA(A) receptors by neurosteroid dehydroepiandrosterone sulfate. *Pharmacology*. 2012;89(3–4):163-171. doi:10.1159/000336058
22. Gravanis A, Calogeropoulou T, Panoutsakopoulou V, Thermos K, Neophytou C, Charalampopoulos I. Neurosteroids and microneurotrophins signal through NGF receptors to induce prosurvival signaling in neuronal cells. *Sci Signal*. 2012;5(246):pt8. doi:10.1126/scisignal.2003387
23. Johansson T, Elfverson M, Zhou Q, Nyberg F. Allosteric modulation of the NMDA receptor by neurosteroids in rat brain and the impact of long term morphine administration. *Biochem Biophys Res Commun*. 2010;401(4):504-508. doi:10.1016/j.bbrc.2010.09.073
24. Johansson T, Le Greves P. The effect of dehydroepiandrosterone sulfate and allopregnanolone sulfate on the binding of [(3)H]ifenprodil to the *N*-methyl-D-aspartate receptor in rat frontal cortex membrane. *J Steroid Biochem Mol Biol*. 2005;94(1–3):263-266. doi:10.1016/j.jsbmb.2005.01.020
25. Noda Y, Kamei H, Kamei Y, Nagai T, Nishida M, Nabeshima T. Neurosteroids ameliorate conditioned fear stress: an association with sigma receptors. *Neuropsychopharmacology*. 2000;23(3):276-284. doi:10.1016/S0893-133X(00)00103-2
26. Mehta AK, Ticku MK. Unsulfated and sulfated neurosteroids differentially modulate the binding characteristics of various radioligands of GABA(A) receptors following chronic ethanol administration. *Neuropharmacology*. 2001;40(5):668-675. doi:10.1016/S0028-3908(00)00200-8
27. Barbosa AD, Morato GS. Influence of neurosteroids on the development of rapid tolerance to ethanol in mice. *Eur J Pharmacol*. 2001;431(2):179-188. doi:10.1016/S0014-2999(01)01337-1
28. Melchior CL, Ritzmann RF. Dehydroepiandrosterone is an anxiolytic in mice on the plus maze. *Pharmacol Biochem Behav*. 1994;47(3):437-441. doi:10.1016/0091-3057(94)90140-6

29. Sharma AN, Chopde CT, Hirani K, Kokare DM, Ugale RR. Chronic progesterone treatment augments while dehydroepiandrosterone sulphate prevents tolerance to ethanol anxiolysis and withdrawal anxiety in rats. *Eur J Pharmacol*. 2007;567(3):211-222. doi:10.1016/j.ejphar.2007.04.025
30. Frias J, Torres JM, Miranda MT, Ruiz E, Ortega E. Effects of acute alcohol intoxication on pituitary-gonadal axis hormones, pituitary-adrenal axis hormones, beta-endorphin and prolactin in human adults of both sexes. *Alcohol Alcohol*. 2002;37(2):169-173. doi:10.1093/alcalc/37.2.169
31. Fukui M, Kitagawa Y, Nakamura N, Kadono M, Hasegawa G, Yoshikawa T. Association between alcohol consumption and serum dehydroepiandrosterone sulphate concentration in men with Type 2 diabetes: a link to decreased cardiovascular risk. *Diabet Med*. 2005;22(10):1446-1450. doi:10.1111/j.1464-5491.2005.01629.x
32. Mahabir S, Baer DJ, Johnson LL, et al. The effects of moderate alcohol supplementation on estrone sulfate and DHEAS in postmenopausal women in a controlled feeding study. *Nutr J*. 2004;3:11. doi:10.1186/1475-2891-3-11
33. Rinaldi S, Peeters PH, Bezemer ID, et al. Relationship of alcohol intake and sex steroid concentrations in blood in pre- and post-menopausal women: the European Prospective Investigation into Cancer and Nutrition. *Cancer Causes Control*. 2006;17(8):1033-1043. doi:10.1007/s10552-006-0041-7
34. Rohwer RD, Liu S, You NC, Buring JE, Manson JE, Song Y. Interrelationship between alcohol intake and endogenous sex-steroid hormones on diabetes risk in postmenopausal women. *J am Coll Nutr*. 2015;34(4):273-280. doi:10.1080/07315724.2014.926163
35. Sierksma A, Sarkola T, Eriksson CJ, van der Gaag MS, Grobbee DE, Hendriks HF. Effect of moderate alcohol consumption on plasma dehydroepiandrosterone sulfate, testosterone, and estradiol levels in middle-aged men and postmenopausal women: a diet-controlled intervention study. *Alcohol Clin Exp Res*. 2004;28(5):780-785. doi:10.1097/01.ALC.0000125356.70824.81
36. Watts EL, Appleby PN, Albanes D, et al. Circulating sex hormones in relation to anthropometric, sociodemographic and behavioural factors in an international dataset of 12,300 men. *PLoS ONE*. 2017;12(12):e0187741. doi:10.1371/journal.pone.0187741
37. Kokavec A, Crowe SF. The effect of a moderate level of white wine consumption on the hypothalamic-pituitary-adrenal axis before and after a meal. *Pharmacol Biochem Behav*. 2001;70(2–3):243-250. doi:10.1016/S0091-3057(01)00606-2
38. Dorgan JF, Reichman ME, Judd JT, et al. The relation of reported alcohol ingestion to plasma levels of estrogens and androgens in premenopausal women (Maryland, United States). *Cancer Causes Control*. 1994;5(1):53-60. doi:10.1007/BF01830726
39. Hirko KA, Spiegelman D, Willett WC, Hankinson SE, Eliassen AH. Alcohol consumption in relation to plasma sex hormones, prolactin, and sex hormone-binding globulin in premenopausal women. *Cancer Epidemiol Biomarkers Prev*. 2014;23(12):2943-2953. doi:10.1158/1055-9965.EPI-14-0982
40. Kokavec A, Lindner AJ, Ryan JE, Crowe SF. Ingesting alcohol prior to food can alter the activity of the hypothalamic–pituitary–adrenal axis. *Pharmacol Biochem Behav*. 2009;93(2):170-176. doi:10.1016/j.pbb.2009.05.004
41. Välimäki MJ, Laitinen K, Tiitinen A, Steman UH, Ylostalo P. Gonadal function and morphology in non-cirrhotic female alcoholics: a controlled study with hormone measurements and ultrasonography. *Acta Obstet Gynecol Scand*. 1995;74(6):462-466. doi:10.3109/00016349509024410
42. Ozsoy S, Esel E. Hypothalamic-pituitary-adrenal axis activity, dehydroepiandrosterone sulphate and their relationships with aggression in early and late alcohol withdrawal. *Prog Neuropsychopharmacol Biol Psychiatry*. 2008;32(2):340-347. doi:10.1016/j.pnpbp.2007.08.034
43. Adinoff B, Kiser JM, Martin PR, Linnoila M. Response of dehydroepiandrosterone to corticotropin-releasing hormone stimulation in alcohol-dependent subjects. *Biol Psychiatry*. 1996;40(12):1305-1307. doi:10.1016/S0006-3223(96)00381-2
44. Heinz A, Weingartner H, George D, Hommer D, Wolkowitz OM, Linnoila M. Severity of depression in abstinent alcoholics is associated with monoamine metabolites and dehydroepiandrosterone-sulfate concentrations. *Psychiatry Res*. 1999;89(2):97-106. doi:10.1016/S0165-1781(99)00099-2
45. Hill M, Popov P, Havlikova H, et al. Altered profiles of serum neuroactive steroids in premenopausal women treated for alcohol addiction. *Steroids*. 2005;70(8):515-524. doi:10.1016/j.steroids.2005.02.013
46. Marx CE, Trost WT, Shampine L, et al. Neuroactive steroids, negative affect, and nicotine dependence severity in male smokers. *Psychopharmacology (Berl)*. 2006;186(3):462-472. doi:10.1007/s00213-005-0226-x
47. Braun B, Weinland C, Kornhuber J, Lenz B. Religiosity, guilt, altruism and forgiveness in alcohol dependence: results of a cross-sectional and prospective cohort study. *Alcohol Alcohol*. 2018;53(4):426-434. doi:10.1093/alcalc/agy026
48. Gegenhuber B, Weinland C, Kornhuber J, Mühle C, Lenz B. OPRM1 A118G and serum beta-endorphin interact with sex and digit ratio (2D:4D) to influence risk and course of alcohol dependence. *Eur Neuropsychopharmacol*. 2018;28(12):1418-1428. doi:10.1016/j.euroneuro.2018.09.002
49. Kalinichenko LS, Mühle C, Jia T, et al. Neutral sphingomyelinase mediates the co-morbidity trias of alcohol abuse, major depression and bone defects. *Mol Psychiatry*. 2021. doi:10.1038/s41380-021-01304-w
50. Lenz B, Köllner MG, Mühle C, Weinland C, Kornhuber J. Basic human body dimensions relate to alcohol dependence and predict hospital readmission. *J Clin Med*. 2019;8(12). doi:10.3390/jcm8122076
51. Lenz B, Weinland C, Bach P, et al. Oxytocin blood concentrations in alcohol use disorder: a cross-sectional, longitudinal, and sex-separated study. *Eur Neuropsychopharmacol*. 2021;51:55-67. doi:10.1016/j.euroneuro.2021.04.015
52. Mühle C, Weinland C, Gulbins E, Lenz B, Kornhuber J. Peripheral acid sphingomyelinase activity is associated with biomarkers and phenotypes of alcohol use and dependence in patients and healthy controls. *Int J Mol Sci*. 2018;19(12). doi:10.3390/ijms19124028
53. Müller CP, Mühle C, Kornhuber J, Lenz B. Sex-dependent alcohol instrumentalization goals in non-addicted alcohol consumers versus patients with alcohol use disorder: longitudinal change and outcome prediction. *Alcohol Clin Exp Res*. 2021;45(3):577-586. doi:10.1111/acer.14550
54. Weinland C, Braun B, Mühle C, Kornhuber J, Lenz B. Cloninger type 2 Score and Lesch typology predict hospital readmission of female and male alcohol-dependent inpatients during a 24-month follow-up. *Alcohol Clin Exp Res*. 2017;41(10):1760-1767. doi:10.1111/acer.13468
55. Weinland C, Mühle C, Kornhuber J, Lenz B. Body mass index and craving predict 24-month hospital readmissions of alcohol-dependent in-patients following withdrawal. *Prog Neuropsychopharmacol Biol Psychiatry*. 2019;90:300-307. doi:10.1016/j.pnpbp.2018.11.019
56. Weinland C, Mühle C, Kornhuber J, Lenz B. Crossed eye/hand laterality and left-eyedness predict a positive 24-month outcome in alcohol-dependent patients. *Alcohol Clin Exp Res*. 2019;43(6):1308-1317. doi:10.1111/acer.14051
57. Weinland C, Tanovska P, Kornhuber J, Mühle C, Lenz B. Serum lipids, leptin, and soluble leptin receptor in alcohol dependence: a cross-sectional and longitudinal study. *Drug Alcohol Depend*. 2020;209:107898. doi:10.1016/j.drugalcdep.2020.107898
58. Anton RF, Moak DH, Latham PK. The obsessive compulsive drinking scale: a new method of assessing outcome in alcoholism

treatment studies. *Arch Gen Psychiatry*. 1996;53(3):225-231. doi:10.1001/archpsyc.1996.01830030047008
59. Mann K, Ackermann K. Die OCDS-G: psychometrische kennwerte der Deutschen version der Obsessive Comulsive Drinking Scale. *Sucht*. 2000;46(2):90-100. doi:10.1024/suc.2000.46.2.90
60. Tannenbaum C, Ellis RP, Eyssel F, Zou J, Schiebinger L. Sex and gender analysis improves science and engineering. *Nature*. 2019;575(7781):137-146. doi:10.1038/s41586-019-1657-6
61. Ylikorkala O, Stenman UH, Halmesmaki E. Testosterone, androstenedione, dehydroepiandrosterone sulfate, and sex-hormone-binding globulin in pregnant alcohol abusers. *Obstet Gynecol*. 1988;71(5):731-735.
62. Hichar JK. Effects of gamma-aminobutyric acid and picrotoxin on spontaneous activity in the central nervous system of the crayfish. *Nature*. 1960;188:1117-1119. doi:10.1038/1881117b0
63. Van Der Kloot WG, Robbins J. The effects of gamma-aminobutyric acid and picrotoxin on the junctional potential and the contraction of crayfish muscle. *Experientia*. 1959;15(1):35-36. doi:10.1007/BF02157093
64. Heinz A, Kiefer F, Smolka MN, et al. Addiction Research Consortium: losing and regaining control over drug intake (ReCoDe)—from trajectories to mechanisms and interventions. *Addict Biol*. 2020;25(2):e12866. doi:10.1111/adb.12866

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Weinland C, Mühle C, von Zimmermann C, Kornhuber J, Lenz B. Sulphated dehydroepiandrosterone serum levels are reduced in women with alcohol use disorder and correlate negatively with craving: A sex-separated cross-sectional and longitudinal study. *Addiction Biology*. 2022;e13135. doi:10.1111/adb.13135