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# Correlations between sex-related hormones, alcohol dependence and alcohol craving

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ABSTRACT

*Background:* Sex-related differences in the susceptibility, progression, and treatment response in alcohol-dependent subjects have been repeatedly reported. In this study, we aimed to investigate the associations of the sex-related hormone/protein levels with alcohol dependence (AD) and alcohol craving in male and female subjects.

*Methods:* Plasma sex-related hormones (estradiol, estrone, total testosterone, progesterone, follicle stimulated hormone [FSH], luteinizing hormone), and sex hormone binding globulin were measured by mass spectrometry or automated immunoassays from 44 recently-abstained subjects (29 males and 15 females; mean age = 45.9 ± 15.6) meeting DSM-IV-TR criteria for AD and 44 age-, sex- and race-matched non-AD controls. Conditional logistic regression was conducted to examine the association of sex-related hormone and protein levels with AD risk, accounting for matching variables. Their associations with alcohol craving scales (Penn Alcohol Craving Scale and Inventory of Drug-Taking Situations) were assessed in AD subjects.

*Results:* Plasma FSH level was significantly higher in AD males (10.3 ± 9.8 IU/L) than control males (8.0 ± 15.9 IU/L; $p = 0.005$, $p_{corrected} = 0.035$). We also found a significant inverse correlation of FSH level with propensity to drink in negative emotional situations (Spearman's rho = -.540; $p = 0.021$) and positive correlations between progesterone level and craving intensity (Spearman's rho = .464; $p = 0.020$) and between total testosterone level and propensity to drink under temptations (adjusted for no-drinking days; $\beta = 6.496$; $p = 0.041$) in AD males.

*Conclusions:* These results suggest that FSH, progesterone, and testosterone levels may be associated with AD and alcohol craving in AD males. Future research is needed to replicate these findings and investigate the underlying biological mechanisms.

## 1. Introduction

Sex-related differences in the susceptibility, progression, and comorbidities of alcoholism are well-known. For example, human males tend to consume more alcohol and engage in heavy drinking more frequently (Wilsnack et al., 2009), have higher prevalence of alcohol use disorder (AUD; Goldstein et al., 2012; Grant et al., 2015), and endorse more alcohol withdrawal symptoms, particularly seizures and anxiety (Deshmukh et al., 2003; Wojnar et al., 1997). Despite consuming less alcohol, females have a higher concentration of alcohol per body weight and progress faster to severe stages of alcoholism (Frezza et al., 1990; Wojnar et al., 1997), are more likely to engage in heavy drinking when experiencing unpleasant emotions or to relieve psychological stress (Lau-Barraco et al., 2009; Rubonis et al., 1994), and experience more severe and persistent depression and anxiety symptoms during withdrawal (Giorgi et al., 2015; Petit et al., 2017). While the role of psychosocial factors (gender-related) seemed to become less significant in differentiating male and female drinkers in recent generations, the impact of biological factors (sex-related) has remained unchanged over time (Erol and Karpyak, 2015). Our research findings indicate that biological (sex-related) factors, including sequence variation in the *PDYN* gene, which encodes the preproprotein prodynorphin (PDYN) and whose truncated products act on kappa opioid receptor (KOR) to mediate aversion (Bazov et al., 2018), may play a role in vulnerability to alcohol dependence and related phenotypes, including propensity to drink in negative emotional states, i.e.,

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negative craving (Karpyak et al., 2013; Preuss et al., 2013). Moreover, our subsequent analyses indicate that these associations are sex-specific and phenotype-dependent (Winham et al., 2015). Earlier studies also report that women tend to drink more in response to negative emotions while men drink more in response to positive emotions (Erol and Karpyak, 2015). Although biological mechanisms underlying these associations remain poorly understood, it is reasonable to assume that sex-related hormones may play a role in those associations.

Sex hormones (estrogens, testosterone, progestins) influence alcohol consumption and vice versa (reviewed by Erol et al., 2019; Lenz et al., 2012a,b). Elevated testosterone levels were associated with increased risk of alcohol use and AUD in men, with a few conclusive findings in women; elevated estrogen levels were associated with increased alcohol use in women, with mixed findings in men (reviewed by Erol et al., 2019). Animal studies also demonstrated that estrogen and progestin increase ethanol intake and drug seeking behaviors in females and males, respectively (reviewed by Carroll and Smethells, 2016; Erol et al., 2019), but testosterone showed suppressive effects in male rats (Bertholomey and Torregrossa, 2017; Vetter-O'Hagen et al., 2011) contrasting to findings in humans. The sex-specificity of these associations implies that sex hormones could underlie the sex differences in the risk of AUD. Moreover, chronic excessive alcohol consumption is also known to impose detrimental effects on fertility and sex hormone levels, which may be caused by its toxic effects on the gonads and liver (Burra, 2013; Van Thiel et al., 1983a). It can result in hypogonadism, sexual dysfunction, and hepatic feminization in males (e.g., Myking et al., 1987; Van Thiel et al., 1975, 1983b) and menstrual abnormalities in females (Augustynska et al., 2007; Li et al., 2013). The levels of sex hormone regulation factors, including gonadotropins (follicle stimulating hormone [FSH], luteinizing hormone [LH]) and sex hormone binding globulin (SHBG), have been shown to differ in alcohol-dependent subjects compared to non-dependent controls (Bertello et al., 1983; Castilla-Garcia et al., 1987; Iranmanesh et al., 1988; Iturriaga et al., 1995; Ruusa et al., 1997), suggesting the upstream regulation of sex hormone production is also altered by excessive alcohol use.

Understanding the sex-specific differences in biological mechanisms of AUD is a critical step towards the development of personalized treatment recommendations. Converging evidence indicates that sex hormones and related regulators may play a pivotal role in alcohol use severity and the development of AUD. However, little is known about the specific role of sex hormones and their regulators in AUD-related phenotypes such as craving, which may underlie sex-specific vulnerability to relapse. In this study, we utilized previously collected plasma samples and clinical information along with community biobank resources to explore sex-specific associations between sex-related hormone/protein levels and alcohol dependence as well as the correlations between sex-related hormone/protein levels and alcohol craving in male alcohol-dependent subjects. Based on previous studies, we hypothesized a higher level of testosterone and a positive association of progesterone with alcohol dependence and/or craving in males, while a positive association between estrogen and alcohol dependence in females. We also hypothesized that in alcohol-dependent individuals, FSH and LH might be dysregulated and SHBG might be reduced.

## 2. Materials and methods

### 2.1. Subjects

This study was approved by the Institutional Review Board of Mayo Clinic, Rochester, and was conducted according to the Declaration of Helsinki. Treatment-seeking alcohol-dependent (AD) subjects were recruited as part of previous studies described elsewhere (Karpyak et al., 2013). All subjects provided written permission to use collected samples and related clinical and demographic information in future studies. Subjects selected for this study fulfilled the criteria for alcohol dependence of the Diagnostic and Statistical Manual of Mental Disorders 4th edition Text Revision (DSM-IV-TR) screened by board-certified addiction psychiatrists and were not currently using sex hormone interfering medication (e.g., oral contraceptives, hormone replacement therapy). Subjects were interviewed and blood samples were drawn shortly after being admitted into residential or outpatient treatment programs affiliated with Mayo Clinic Addiction Services in Rochester, Minnesota. Standard demographic and clinical information were collected including alcohol craving scales described below. Based on the availability of sufficient amounts of plasma samples, 44 AD subjects were included in this study (15 females with mean age of 45.0 ± 13.4 years; 29 males with mean age of 46.1 ± 16.9 years). Forty-four control subjects (15 females with mean age of 45.0 ± 13.4 years; 29 males with mean age of 46.4 ± 16.9 years), who reported never having alcohol use-related problems and were not currently taking oral contraceptives or other sex hormone therapy (see Supplementary Materials for the list of excluded sex hormone interfering medications), were selected from the Mayo Clinic Biobank (Olson et al., 2013) and were individually-matched to the AD subjects on sex, age and ethnicity.

### 2.2. Alcohol craving scales

Penn Alcohol Craving Scale (PACS; Flannery et al., 1999) is a 5-item self-report questionnaire focusing on the frequency, intensity, and duration of craving as well as the disability to resist drinking over the past week. Inventory of Drug-Taking Situations (IDTS; Annis et al., 1997) is a 50-item self-report questionnaire identifying the propensity for drinking in various high-risk situations including positive and negative emotional states or presence of strong temptations. As recommended (Turner et al., 1997), we averaged the item scores under unpleasant emotions, physical discomfort, and conflict with others as "negative craving"; those under pleasant emotions, testing personal control, social pressure to use, and pleasant times with others as "positive craving"; and those under urges and temptations as "temptation".

### 2.3. Sample collection

Peripheral venous blood was sampled into EDTA-coated tubes from the AD subjects in early abstinence (median days between admission and blood draw = 15.5 days) and from the controls on normal days. Blood samples were centrifuged at 2000 *g* for 10 min. Plasma samples were extracted, aliquoted and stored at −80 °C until use. 1 mL of plasma sample from each subject was sent to the Mayo Clinic Immunochemistry Laboratory for sex-related hormone/protein quantitation. Staff members performing the quantification were blinded to the group or sex identity of the samples.

### 2.4. Measurement of estrogens

Estrogens (17-β-estradiol and estrone) were first extracted from the plasma samples with methylene chloride and then derivatized with dansyl chloride. The derivatized sample extracts were then processed through high-pressure liquid chromatography (HPLC) followed by detection with tandem mass spectrometry (MS/MS; Agilent Technologies, Inc., Santa Clara, CA, USA). Intra-assay coefficients of variation (CVs) of estradiol were 11.8%, 7.3%, 6.0%, 1.6%, 1.5% and 1.4% at 0.23, 0.50, 0.74, 35, 151 and 405 pg/mL, respectively; inter-assay CVs were 10.8%, 8.8%, 6.9%, 5.1%, 4.6% and 4.8% at 0.29, 0.50, 0.77, 32, 140 and 382 pg/mL, respectively. For estrone, intra-assay CVs were 17.8%, 7.5%, 6.1%, 2.5%, 1.7% and 1.2% at 0.30, 0.50, 0.84, 32, 142 and 389 pg/mL, respectively; inter-assay CVs were 12.0%, 9.5%, 7.9%, 7.4%, 7.1% and 6.6% at 0.25, 0.51, 0.85, 30, 131 and 355 pg/mL, respectively.

### 2.5. Measurement of testosterone

Total testosterone was measured by liquid chromatography coupled

with tandem mass spectrometry (LC–MS/MS; Agilent Technologies, Santa Clara, CA, USA). Intra-assay CVs of total testosterone were 7.4%, 6.1%, 9.0%, 2.3% and 0.9% at 0.65, 4.3, 48, 118 and 832 ng/dL, respectively; inter-assay CVs were 8.9%, 6.9%, 4.0%, 3.6% and 3.5% at 0.69, 4.3, 45, 117 and 841 ng/dL, respectively.

### 2.6. Measurement of progesterone

Progesterone was measured by competitive immunoenzymatic assays performed on Roche Cobas e411 Analyzer (Roche Diagnostics, Indianapolis, IN, USA). Inter-assay CVs of progesterone were 4.8%, 2.2%, and 1.8% at 0.474, 8.26 and 28.5 ng/mL, respectively.

### 2.7. Measurement of FSH and LH

FSH and LH were measured by two-site immunoenzymatic assays performed on UniCel DxI 800 Immunoassay System (Beckman Coulter, Inc., Chaska, MN, USA). Intra-assay CVs of FSH were 3.2% and 2.8% at 8.6 and 47.1 mIU/mL, respectively; inter-assay CVs were 3.6%, 3.2%, and 4.7% at 6.5, 16.7, and 58.0 mIU/mL, respectively. For LH, intra-assay CVs were 4.3% and 4.0% at 1.2 and 38.5 mIU/mL, respectively; inter-assay CVs were 9.3%, 6.0%, and 6.0% at 1.4, 15.6, and 48.8 mIU/mL, respectively.

### 2.8. Measurement of SHBG

SHBG was measured by a two-site chemiluminescent assay performed on IMMULITE 2000 (Siemens Healthcare Diagnostic, Deerfield, IL, USA). Intra-assay CVs of SHBG were 2.7% and 3.1% at 5.5, and 95.9 nmol/L, respectively; inter-assay CVs were 4.0% and 5.9% at 5.4 and 74 nmol/L, respectively.

### 2.9. Statistical analysis

Statistical analyses were performed in SAS version 9.4 (Cary, NC, USA). Descriptive statistics were computed to summarize the AD cases and matched controls. Spearman's correlation coefficients for each hormone level and age were calculated overall and separately in cases and controls. For each sex hormone level, matched case and control pairs were compared with paired *t*-tests or Wilcoxon signed-rank tests, as appropriate based on the data distribution. Age-adjusted analyses were also conducted with conditional logistic regression. Within the cases, linear models were fit to compare sex hormone levels and craving scales, separately for PACS, IDTS negative, positive, and temptation craving scores; models were adjusted for age. All analyses were conducted separately for males and females. Statistical significance was established at $p < 0.05$ and multiple testing-corrected $p$ threshold was $0.05 \div 7 = 0.0071$ (Bonferroni method).

## 3. Results

### 3.1. Demographic and alcohol-related characteristics of male and female alcohol-dependent subjects

No significant differences were found between sexes in the number of days between the last drink and blood draw or scores on various craving scales (Table 1). Two male subjects and one female subject were diagnosed with alcoholic liver disease prior to the date of blood draw, so sensitivity analyses were conducted excluding these samples and results remained unchanged.

### 3.2. Correlation with age and differences in sex-related hormone/protein levels between sexes

The correlations between age and the tested sex-related hormones/protein levels are shown in Supplementary Table S1 and the correlations among the sex-related hormones/protein levels are shown in Supplementary Table S2. In female subjects overall, age was significantly positively correlated with the levels of FSH and LH (Spearman's rho = .735 and .581, $p < 0.001$) but negatively correlated with the level of progesterone (Spearman's rho = -.380, $p = 0.039$); the levels of estradiol and SHBG also trended towards negative correlations with age ($p < 0.10$). In male subjects overall, age was significantly positively correlated with FSH and SHBG levels (Spearman's rho = .406 and .331, $p < 0.05$) but negatively correlated with progesterone level (Spearman's rho = $-.513$, $p < 0.001$). None of the sex-related hormone/protein levels were significantly correlated with the number of days from last drink ($p > 0.05$) in female AD subjects; while LH levels demonstrated a positive correlation with the number of days from last drink in male AD subjects (Spearman's rho = .420; $p = 0.035$). After controlling for age, female subjects overall had significantly higher levels of estrone, estradiol, FSH, LH, progesterone, and SHBG ($p < 0.05$), while male subjects overall had a significantly higher level of testosterone ($p < 0.05$; Table 2).

### 3.3. Sex-related hormone/protein levels associated with alcohol dependence

Among female subjects, none of the measured hormone/protein levels were significantly different between AD subjects and controls ($p > 0.05$; Table 2), even when female subjects above age 51 (the average age of menopause occurrence; Stuenkel et al., 2015) were removed (Supplementary Table S3). Among male subjects, most of the measured hormone/protein levels were not significantly different between subject groups, apart from FSH, which was significantly higher in the AD subjects ($p = 0.005$; $p_{corrected} = 0.035$; Fig. 1A), and SHBG, which showed a statistical trend for being higher in the AD subjects ($p = 0.072$; Table 2). The FSH finding persisted after sensitivity analyses excluding the subjects with alcoholic liver disease.

Based on previous reports on the effects of abstinence time on sex-related hormone/protein levels, we checked for possible correlations between the tested sex-related hormone/protein levels and the number of days from last drink in the AD subjects. No significant correlations were detected ($p > 0.05$) in female AD subjects; while a positive correlation was detected in male AD subjects (Spearman's rho = 0.420; $p = 0.035$; Supplementary Table S4). The time of last alcohol consumption was not adjusted in the association with AD since related data were absent in controls.

### 3.4. Correlations between sex-related hormone/protein levels and alcohol craving

Due to more than half of the female AUD subjects having missing data on alcohol craving measures, our correlation analyses between sex-related hormones/proteins and alcohol craving were focused on male AUD subjects and the results are presented in Table 3. Among these subjects, we detected a significant inverse correlation between IDTS negative craving score and FSH level (Spearman's rho = $-.540$, $p = 0.021$; Fig. 1B). IDTS negative craving score was also marginally positively correlated with progesterone level (Spearman's rho = .469, $p = 0.050$). We also detected a significant positive correlation between PACS score and progesterone level (Spearman's rho = .464, $p = 0.020$). When the number of days from last drink was adjusted using multivariable linear model (Supplementary Table S5), the significant negative correlation of FSH with IDTS remained and a significant negative correlation between FSH and PACS was appeared ($\beta = -0.262$ and $-0.609$, $p = 0.015$ and 0.027, respectively), while the positive correlation of the progesterone with IDTS negative craving was weakened ($\beta = 0.020$, $p = 0.083$). Meanwhile, a strong significant positive correlation emerged between total testosterone levels and IDTS temptation score ($\beta = 6.496$, $p = 0.041$).

**Table 1**
Subject characteristics.

| | Alcohol Dependent (AD) | | | Controls | | |
|---|---|---|---|---|---|---|
| | Females ($n$ = 15) | Males ($n$ = 29) | $P$ value | Females ($n$ = 15) | Males ($n$ = 29) | $P$ value |
| Age (mean year; SD) | 45.0 (13.4) | 46.1 (16.9) | 0.870 | 45.0 (13.4) | 46.4 (16.9) | 0.785 |
| > 51 years old ($n$; %) | 8 (53.3%) | 12 (41.4%) | 0.450 | 8 (53.3%) | 12 (41.4%) | 0.450 |
| Days between last drink and blood draw (median day; range) | 20 (11 – 261) | 15 (2 – 58) | 0.361[§] | – | – | – |
| PACS (mean score; SD) | 6.0 (5.5) | 10.1 (8.3) | 0.164 | – | – | – |
| IDTS (mean score; SD) | | | | | | |
| Negative emotions[†] | 57.3 (15.5) | 40.0 (24.3) | 0.190 | – | – | – |
| Positive emotions[‡] | 45.3(11.8) | 40.6(28.0) | 0.780 | – | – | – |
| Temptation[#] | 48.5 (6.5) | 31.8 (15.9) | 0.057 | – | – | – |

Note: Control subjects were individually matched to AD subjects.
[§] Mann-Whitney $U$ test.
[†] Based on 4 females and 18 males.
[‡] Based on 3 females and 16 males.
[#] Based on 4 females and 17 males.

**Table 2**
Sex-related hormone/protein levels of alcohol-dependent (AD) subjects and age-and-sex-matched controls.

| Hormone levels; Mean (SD) | Females ($n$ = 30) | | | Males ($n$ = 58) | | | Overall F $vs$ M |
|---|---|---|---|---|---|---|---|
| | Controls ($n$ = 15) | AD ($n$ = 15) | $P$ value | Controls ($n$ = 29) | AD ($n$ = 29) | $P$ value | $P$ value |
| Estrone (pg/mL) | 46.0 (35.4) | 40.6 (28.8) | 0.512 | 27.2 (9.7) | 27.5 (9.6) | 0.905 | < 0.001 |
| Estradiol (pg/mL) | 55.0 (68.7) | 57.4 (84.2) | 0.922 | 17.1 (7.8) | 20.4 (9.1) | 0.213* | 0.011 |
| Testosterone (total; ng/dL) | 16.2 (6.3) | 17.0 (7.7) | 0.769 | 366.6 (177.5) | 418.4 (276.8) | 0.436 | < 0.001 |
| Progesterone (ng/mL) | 2.1 (5.6) | 1.3 (3.3) | 0.685 | 0.5 (0.3) | 0.5 (0.3) | 0.912 | < 0.001 |
| FSH (IU/L) | 23.9 (25.5) | 38.0 (41.1) | 0.157 | 8.0 (15.9) | 10.3 (9.8) | 0.005*[#] | 0.004 |
| LH (IU/L) | 14.6 (13.8) | 21.1 (21.7) | 0.306 | 4.6 (5.1) | 6.9 (10.7) | 0.124* | 0.049 |
| SHBG (nmol/L) | 71.4 (40.3) | 59.9 (25.6) | 0.176 | 29.1 (12.0) | 36.2 (21.2) | 0.072* | < 0.001 |

## 4. Discussion

This study investigated the relationship of plasma sex-related hormone levels with alcohol dependence and alcohol craving characteristics in a cohort of a recently abstained AD subjects and matched controls. As expected, we observed moderate to strong sex-specific correlations between age and the levels of tested sex-related hormones/proteins along with between-sex differences in sex-specific hormone levels after adjustment for age. We also identified a significantly higher plasma FSH level and a trend for increased SHBG level in AD males compared to male controls. We further detected a significant inverse correlation between plasma FSH level and propensity to drink in negative emotional states (measured by IDTS negative craving subscale) along with a significant positive correlation between plasma progesterone level and the intensity of state-dependent craving (measured by PACS score). After adjusting for the days from last drink, a negative correlation between plasma FSH level and state-dependent craving intensity (measured by PACS) and a strong positive correlation between plasma total testosterone level and the propensity to drink when encountering temptations (measured by IDTS temptation subscale) emerged in AD males. These findings represent the first evidence suggesting that the differences in sex-related hormone levels may be associated with AD and related phenotypes in a sex-specific manner. However, it remains to be determined if this involvement is temporary (i.e., state- or trait-dependent), causative (i.e., alcohol use triggers the change of sex-related hormone levels or vice versa), or the combination of both.

Our observation of significantly higher plasma FSH levels in male AD subjects compared to male controls is in agreement with the majority of previous studies conducted in alcoholic males during early abstinence (Bertello et al., 1983; Castilla-Garcia et al., 1987; Iranmanesh et al., 1988; Martinez-Riera et al., 1995; Muthusami and Chinnaswamy, 2005; Ruusa et al., 1997) apart from one which found no significant difference between AD and controls (Iturriaga et al., 1995). FSH elevation had been observed after acute alcohol exposure (Valimaki et al., 1984) or shortly after cessation of chronic drinking (Bertello et al., 1983; Castilla-Garcia et al., 1987; Iranmanesh et al., 1988; Martinez-Riera et al., 1995; Ruusa et al., 1997) in experimental and clinical studies. These findings suggest that the elevation of FSH level may be triggered by a reduction in blood testosterone levels caused by the toxic effects of ethanol on the Leydig cells and the inhibition of enzymes involved in testosterone biosynthesis (Van Thiel et al., 1983a). The early-abstinence-associated elevation of FSH appears to be temporary and gradually returns to normal levels in longer-term (after 3 weeks) with the rise of testosterone level (Ruusa et al., 1997). Considering the variability in abstinence length among our study participants, we performed additional analyses and found that the number of days from last drink did not associate with FSH in male AD subjects (Supplementary Table S4), thus supporting that elevated plasma FSH could be a trait characteristic associated with heavy alcohol use rather than one of its consequences. It is also important to mention that in FSH levels do not always correspond to elevated testosterone levels in non-cirrhotic AD subjects compared to non-alcoholic controls (Hasselblatt et al., 2003; Martinez-Riera et al., 1995). Similarly, we did not observe a significant difference in plasma total testosterone level in male AD subjects and controls. Although the free (bioavailable) testosterone level was not measured in our study, the marginally increased plasma SHBG level in the AD subjects might imply a relatively lower free testosterone level. Alternatively, these results might suggest that our male AD subjects may not have testosterone insufficiency, and hence the increased FSH level in this group may be of a different etiology.

Another possible explanation could involve mechanisms outside the

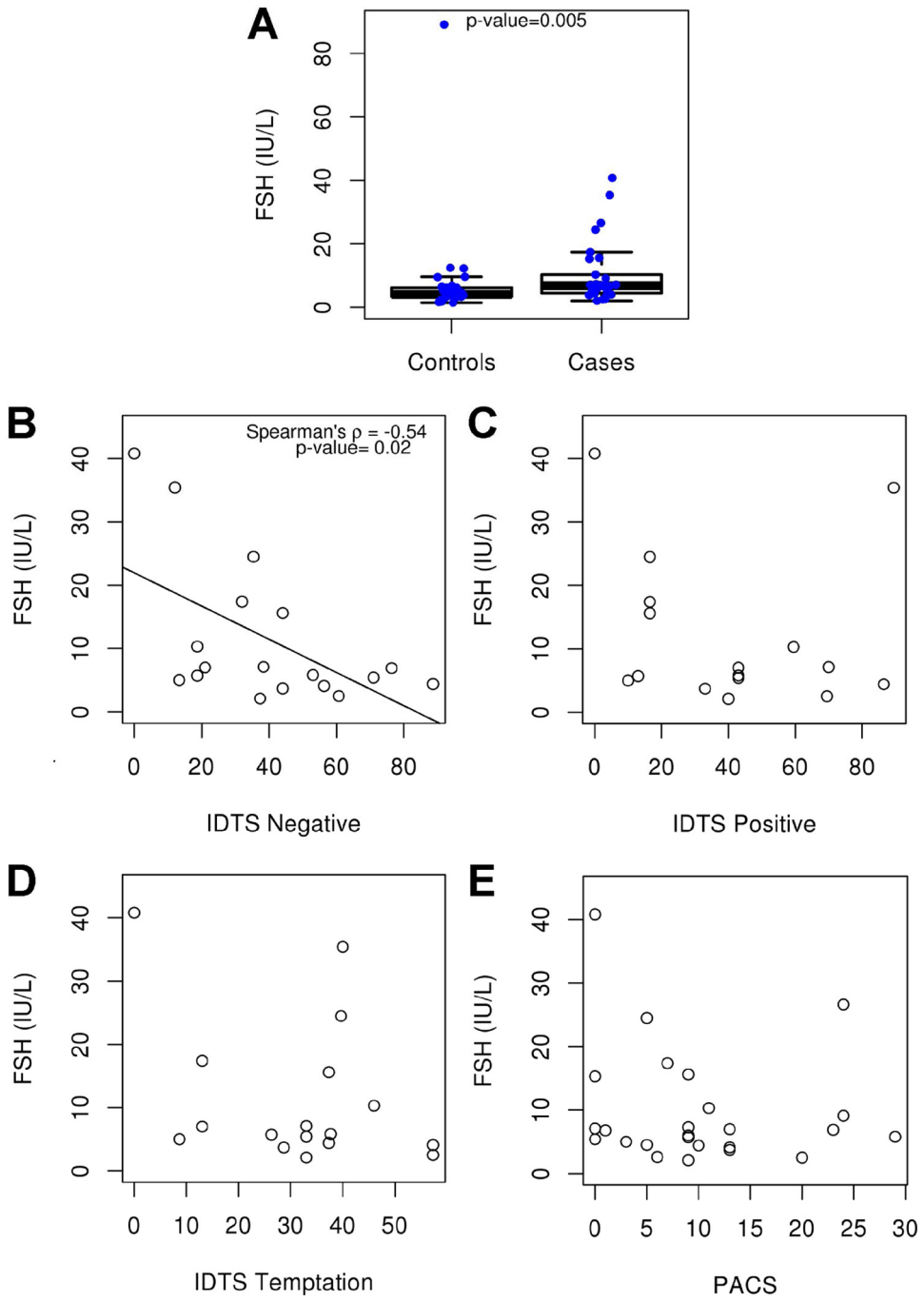


**Fig. 1.** Plasma FSH level was significantly higher in male alcohol-dependent (AD) subjects compared to male controls (A). In male AD subjects, plasma FSH level was negatively correlated with IDTS negative craving score (B), but was not correlated with the scores of IDTS positive craving (C), temptation (D), and PACS (E).

negative feedback loop regulating the release of FSH and LH in the anterior pituitary controlled by gonadotropin-releasing hormone (GnRH) produced in the hypothalamus in response to peripheral testosterone level such as the PDYN/KOR system. Dynorphin (Dyn; a truncated product of PDYN) produced by the kisspeptin/neurokinin/dynorphin (KNDy) neurons acts through KOR to inhibit kisspeptin secretion onto GnRH neurons to precisely control GnRH pulsatility (Navarro, 2012). The PDYN/KOR system was found to be downregulated in the dorsolateral prefrontal cortex and dorsal striatum or dysregulated in the nucleus accumbens of alcoholic individuals (Bazov et al., 2018). Sex-specific action in the PDYN/KOR system was observed. In male rats but not in female rats, KOR antagonist pretreatment increased the circulating levels of FSH and LH as well as their responses (Ruiz-Pino et al., 2015). The gene expression levels of *Pdyn* and *Opkr1* in the mediobasal hypothalamus was lower in male prepubertal rats than their female counterparts (Ruiz-Pino et al., 2015). We also found the association between *PDYN* gene polymorphisms and alcohol dependence in male AD subjects (Winham et al., 2015). Therefore, our observation of increased FSH levels in male AD subjects may be related to a downregulated PDYN/KOR tone and the gene polymorphisms in genes involved in the PDYN/KOR system.

We found a negative correlation between FSH level and state-dependent craving intensity (measured by PACS) after adjusting for days from last drink in male AD subjects. Similarly, we also observed an inverse correlation between FSH level and IDTS negative craving scores in male AD subjects (i.e., AD males with higher FSH level experienced

**Table 3**
Correlations (Spearman's rho with *p*-values in brackets) between the levels of sex-related hormones/proteins and alcohol craving scales in male alcohol-dependent subjects.

| | PACS | IDTS Negative Craving |
|---|---|---|
| Estrone | .216 (0.300) | −.125 (0.621) |
| Estradiol | .242 (0.244) | .131 (0.606) |
| Total Testosterone | .373 (0.066) | .343 (0.164) |
| Progesterone | .464 (0.020*) | .469 (0.050) |
| FSH | −.143 (0.494) | −.540 (0.021*,#) |
| LH | .103 (0.623) | −.212 (0.400) |
| SHBG | .067 (0.750) | −.400 (0.103) |

FSH: follicle stimulating hormone; LH: luteinizing hormone; SHBG: sex hormone binding globulin.

* *p*-value < 0.05.

\# After exclusion of three subjects with alcoholic liver disease diagnosis: Spearman's rho = -.620 and $p = 0.007$.

milder alcohol craving under negative emotions) which was independent of days from last drink. These findings suggest that FSH might be involved in the modulation of state-dependent craving and negative emotions-related craving in AD men, highlighting the importance of considering different aspects of craving phenomenology (i.e., intensity and the context of the situations during which craving intensifies). Despite the scarcity of literature demonstrating FSH's influence on emotion and alcohol craving in males, the possibility of FSH levels being affected by emotional dysregulation should not be ignored. For instance, FSH and LH levels were found to be positively associated with self-reported depression and anxiety levels in infertile men (Bak et al., 2012; Bhongade et al., 2015). Future research is needed to clarify the role of FSH in emotion and craving regulation, as well as whether this effect is male-specific.

In addition to FSH, total testosterone levels in male AD subjects were positively correlated with IDTS temptation score after adjusting for the number of days from last drink. In other words, AD male subjects with higher total testosterone levels perceived stronger alcohol craving under temptation situations, e.g., drinking-related environments, social pressure, testing personal control. This correlation concurs with multiple reports of positive associations between testosterone level and alcohol consumption observed in human males (reviewed by Erol et al., 2019). A longitudinal study following early-abstained AD male patients observed a significantly higher serum testosterone level compared to control males and a positive correlation between the decrease of serum testosterone levels from day 1 to 7 and alcohol craving level on day 7 (Heberlein et al., 2016), suggesting that higher testosterone levels are associated with stronger alcohol craving during abstinence. It should be noted that such correlation was not observed in experiments with castrated rats which demonstrated that testosterone treatment suppressed ethanol intake (Bertholomey and Torregrossa, 2017; Vetter-O'Hagen et al., 2011). However, castration can significantly reduce neural androgen receptors in 6 h (Cunningham et al., 2012), androgen sensitivity in the brains of castrated animals without testosterone replacement may be altered compared to that of intact animals (Antonio et al., 1999), thus the duration between castration and hormonal replacement needs to be considered.

This is also the first study to report a positive correlation between progesterone level and state-dependent alcohol craving (measured by PACS) in male AD subjects. Progesterone metabolites allopregnanolone and pregnanolone are neuroactive steroids that act on the $GABA_A$ receptor as positive allosteric modulators — alcohol is also a $GABA_A$ receptor positive allosteric modulator (Belelli and Lambert, 2005). Manipulating the levels of these progesterone metabolites was found to influence alcohol-withdrawal effects in both animal and human studies; in particular, higher levels or elevation of these metabolite levels were associated with alleviation of alcohol withdrawal effects (reviewed by Anker and Carroll, 2010), which could be attributed to their anxiolytic and anticonvulsant properties (Anker and Carroll, 2010). Administration of allopregnanolone reduced ethanol self-administration in rodents subjected to prolonged ethanol exposure (Ford et al., 2005; Martin-Garcia et al., 2007) while blocking allopregnanolone synthesis reduced ethanol-seeking behavior (Ford et al., 2008), thus implicating progesterone metabolites in the regulation of alcohol craving. Our observation of a positive correlation between progesterone and recent alcohol craving level appears to contradict this notion since a positive relationship between the levels of progesterone and its metabolites were expected. Such discordance may be caused by the inefficient conversion of progesterone to its metabolites. Chronic alcohol use had been shown to reduce hepatic 5α-reductase activity in cases of alcoholic liver disease (Gordon et al., 1979). A genetic study reported association between the genes of key enzymes for generating endogenous neuroactive steroids (5α-reductase 1 and 2 and 3α-hydroxysteroid dehydrogenase 2) and alcohol dependence (Lenz et al., 2012a,b; Milivojevic et al., 2011), and one of the protective alleles discovered had been associated with higher 5α-reductase activity (Ellis et al., 2005), suggesting that AD individuals might be more likely to have reduced activity of the enzyme and in turn attenuating progesterone metabolite biosynthesis and accumulating progesterone. Nevertheless, the progesterone levels in our male subjects were much lower than the female subjects and its correlation with PACS score weakened after adjusting for the number of days from last drink. Hence, the observed significant correlation needs to be taken cautiously and requires further investigation.

The lack of significant differences in sex-related hormone/protein levels, particularly estradiol, between female AD subjects and female controls was unexpected since there is copious evidence from human and animal studies suggesting higher estradiol was associated with higher alcohol consumption (Erol et al., 2019). Whilst some of this evidence was based on observational studies by assessing alcohol/ethanol consumption amount and pattern at different menstrual/estrus cycle phases (e.g., Mello et al., 1986; Pastor and Evans, 2003; Sutker et al., 1983), rodent experiments showed chronic estradiol treatment augmented ethanol consumption (Bertholomey and Torregrossa, 2017; Satta et al., 2018); however, human laboratory studies found no change in alcohol consumption after oral estradiol administration in young healthy women (Little et al., 1980). The positive relationship between estradiol level and alcohol consumption has been postulated to be caused by the redox imbalance (increased NADH/NAD ratio) resulting from alcohol degradation which in turn favors the estrone-to-estradiol conversion (Sarkola et al., 1999). Despite Augustynska et al. (2007) reporting no abnormal elevation of estradiol levels in AD premenopausal women at various menstrual cycle phases during the first week of alcohol abstinence, the unknown current menstrual cycle phases of our female subjects might confound our analyses.

A few limitations of this study should be noted. Since this study utilized existing plasma samples from previous studies and community biobank, the investigation of potentially important factors, such as the stage of menstrual cycle, was not possible. We were also unable to control the time and day of blood sampling. Although these particular limitations were unlikely to impact study findings in males, it might have contributed to the failure to detect a significant correlation in female AD subjects where sample size was lower. The lack of craving information in some of the female AD subjects further reduced the sample size hence hindering related analyses. The small sample size restricted our analyses of the correlations between sex-related hormones/proteins and alcohol craving levels in AD females, hence prohibiting us from determining whether the significant observations in AD males were truly sex-specific. The overall small sample size also limited statistical power to perform multivariate modeling with multiple hormones simultaneously and to account for multiple comparisons. However, the difference in FSH levels in male subjects, which survived the stringent Bonferroni-correction, and the promising correlation with craving measures warrant further investigation.

## 5. Conclusions

Our findings provide support for the role of FSH and other sex-related hormones in AD-related phenotypes including the regulation of craving induced by negative emotions in AD males. Consistent with previous reports, we found higher plasma FSH levels in AD males compared to age-matched controls. We also discovered that in AD males, plasma FSH levels inversely correlated with craving intensity and the propensity to drink in negative emotional states, plasma total testosterone levels positively correlated with the propensity to drinking under temptation after adjusting for days from last drink, and plasma progesterone levels correlated positively with carving intensity. Future studies that include larger numbers of AD male and female subjects with prospective data collection are needed to replicate our results and ascertain whether the findings are sex-specific. The inclusion of other factors which are known to modulate sex-related hormone and associated with alcoholism, such as prolactin and ghrelin, may be considered for a comprehensive overview of how these hormone/proteins are regulated.

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## Contributors

AMC, SJW, and VMK conceived of the study. AMC prepared the manuscript and coordinated sample retrieval. SJW and JRG performed subject selection and matching as well as statistical analyses. SJW, GB and VMK provided critical comments on the manuscript. All authors read and approved the final manuscript.

## Conflicts of interest

No conflict declared.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.drugalcdep.2019.01.029.

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