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REGULAR RESEARCH ARTICLE

Neuroendocrine Response to Exogenous Ghrelin Administration, Combined With Alcohol, in Heavy-Drinking Individuals: Findings From a Randomized, Double-Blind, Placebo-Controlled Human Laboratory Study

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Significance Statement

Administration of the hormone ghrelin has been shown to increase alcohol craving and drinking. Therefore, the ghrelin system is being studied as a potential target to develop novel medications to treat alcohol use disorder (AUD). Both ghrelin and alcohol interact with a variety of endocrine pathways, especially those related to appetite, metabolism, and stress. To better understand the complex interplay between ghrelin and other hormones in the context of alcohol use, the present study examined neuroendocrine response to a supraphysiological challenge with exogenous ghrelin, combined with alcohol, in a clinically relevant sample of heavy-drinking individuals with AUD. Results were consistent across 2 experimental alcohol administration paradigms and found a number of endocrine changes in response to exogenous ghrelin administration. This study provides a comprehensive picture of neuroendocrine response to ghrelin plus alcohol and provides a deeper insight into the interplay between ghrelin and appetitive, metabolic, and stress-related hormones in the context of alcohol use.

Abstract

Background: Accumulating evidence has established a role for the orexigenic hormone ghrelin in alcohol-seeking behaviors. Accordingly, the ghrelin system may represent a potential pharmacotherapeutic target for alcohol use disorder. Ghrelin modulates several neuroendocrine pathways, such as appetitive, metabolic, and stress-related hormones, which are particularly relevant in the context of alcohol use. The goal of the present study was to provide a comprehensive assessment of neuroendocrine response to exogenous ghrelin administration, combined with alcohol, in heavy-drinking individuals.
Methods: This was a randomized, crossover, double-blind, placebo-controlled human laboratory study, which included 2 experimental alcohol administration paradigms: i.v. alcohol self-administration and i.v. alcohol clamp. Each paradigm consisted of 2 counterbalanced sessions of i.v. ghrelin or placebo administration. Repeated blood samples were collected during each session, and peripheral concentrations of the following hormones were measured: leptin, glucagon-like peptide-1, pancreatic polypeptide, gastric inhibitory peptide, insulin, insulin-like growth factor-1, cortisol, prolactin, and aldosterone.
Results: Despite some statistical differences, findings were consistent across the 2 alcohol administration paradigms: i.v. ghrelin, compared to placebo, increased blood concentrations of glucagon-like peptide-1, pancreatic polypeptide, cortisol, and prolactin, both acutely and during the whole session. Lower levels of leptin and higher levels of aldosterone were also found during the ghrelin vs placebo session.
Conclusion: These findings, gathered from a clinically relevant sample of heavy-drinking individuals with alcohol use disorder, provide a deeper insight into the complex interplay between ghrelin and appetitive, metabolic, and stress-related neuroendocrine pathways in the context of alcohol use.

Key Words: Ghrelin, alcohol, neuroendocrine, metabolism, stress

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Introduction

Alcohol is the most commonly used addictive drug worldwide, and alcohol use disorder (AUD) represents a major global public health concern (Rehm et al., 2018). Despite the high prevalence of AUD and considerable medical, psychosocial, and economic burden associated with the disease (Grant et al., 2016), treatment options, including medications, are limited in number and efficacy (Jonas et al., 2014). Therefore, there is a critical need to increase the armamentarium of pharmacotherapies for AUD (Farokhnia et al., 2019a). While most of the research in this regard has focused on central neurobiological mechanisms involved in addictive behaviors, there is growing interest in understanding the role of peripheral/modulatory pathways (e.g., endocrine system, immune factors, gut microbiome) and their potential as novel therapeutic targets for AUD (Engel and Jerlhag, 2014; Ray et al., 2014; Temko et al., 2017).

Among drugs with addictive properties, alcohol has some unique characteristics, as it not only exerts pharmacological actions in the central nervous system (CNS) and the periphery but also has palatable properties and is a direct source of calories. In fact, previous research indicates considerable overlap between biological processes involved in food craving, intake, and metabolism and those that regulate alcohol-seeking and consummatory behaviors (Volkow et al., 2012; Blanco-Gandía et al., 2020). Notably, appetitive/metabolic hormones such as ghrelin, leptin, glucagon-like peptide-1 (GLP-1), and insulin that control homeostatic feeding and metabolism have also been found to regulate hedonic and addictive properties of food and alcohol, mainly through interactions with pathways related to reward processing (van Zessen et al., 2012). In addition, several lines of evidence suggest that stress-related pathways modulate both food and alcohol-seeking behaviors, primarily through negative reinforcement mechanisms (Koob et al., 2014). The hypothalamic-pituitary-adrenal (HPA) axis, a key neuroendocrine pathway involved in stress response, is directly activated by alcohol at the pharmacological level (Zhou and Kreek, 2014). Moreover, previous studies indicate that alterations in the HPA axis may facilitate the transition from mild-to-moderate alcohol drinking to AUD and may contribute to the risk of relapse (Koob, 2010; Blaine and Sinha, 2017). Based on the aforementioned evidence, feeding- and stress-related endocrine pathways may represent novel pharmacotherapeutic targets for AUD. One such pathway is the ghrelin system, with growing evidence from preclinical and clinical studies supporting its role in biobehavioral mechanisms underlying alcohol seeking and consumption (Farokhnia et al., 2019b).

Ghrelin is a 28-amino acid peptide hormone primarily produced by enteroendocrine cells located in the oxyntic glands of the stomach. A portion of the produced proghrelin undergoes acylation via the ghrelin-O-acyltransferase enzyme; the acylated peptide is subsequently cleaved to form acyl-ghrelin.

Acyl-ghrelin has been termed the "active" form of ghrelin for its ability to bind to and activate the growth hormone secretagogue receptor 1a (GHSR1a), also known as the ghrelin receptor (Gahete et al., 2014). GHSR1a is a G protein-coupled receptor widely expressed in both central (e.g., hypothalamus, pituitary, ventral tegmental area, amygdala, hippocampus) and peripheral (e.g., gut, pancreas, adipose tissue, adrenal gland, vagus nerve terminals) tissues (Gnanapavan et al., 2002), mediating ghrelin's functions in the CNS and the periphery.

Ghrelin was first discovered to stimulate the release of growth hormone (GH) from the pituitary through GHSR1a activation (Kojima et al., 1999). Subsequent research has identified a wide range of other key physiological functions, indicating that the ghrelin system is critical for survival (Mani and Zigman, 2017). Chiefly, ghrelin regulates both homeostatic and hedonic food intake (Perelló and Zigman, 2012; Yanagi et al., 2018). Ghrelin also plays a major role in energy balance, as it regulates calorie intake and expenditure and modulates key metabolic processes, such as those involved in lipid and glucose homeostasis (Pradhan et al., 2013; Lv et al., 2018; Gray et al., 2019). Growing evidence suggests that ghrelin may also be considered a stress hormone, as it closely interacts with biobehavioral pathways that regulate stress response, for example, the HPA axis (Morris et al., 2018; Stone et al., 2020). Accordingly, the ghrelin system has been extensively studied in relation to alcohol-related behaviors and is currently under investigation as a potential therapeutic target for AUD (Zallar et al., 2017; Farokhnia et al., 2019b; Lee et al., 2020).

Ghrelin's effects on alcohol-related outcomes are thought to be primarily driven through brain regions and neural circuits involved in reward processing, stress regulation, and cognition (Jerlhag et al., 2009; Meyer et al., 2014; Koob and Volkow, 2016). Peripherally secreted ghrelin is able to cross the blood-brain barrier and binds to the GHSR1a in the CNS (Banks, 2012). Another route through which ghrelin interacts with the brain is vagal stimulation via activation of the GHSR1a expressed on vagal afferent neurons (Dockray, 2013; Date, 2014; Tamboli et al., 2017; Godlewski et al., 2019). Given the widespread presence of ghrelin receptors in both central and peripheral tissues, ghrelin signaling modulates a myriad of other neuroendocrine pathways, such as appetitive/metabolic and stress-related hormones. These effects are particularly relevant in the context of alcohol use, as neuroendocrine mechanisms may also be implicated in the pathophysiology of AUD. In addition to a large body of preclinical evidence on the interaction between ghrelin, alcohol, and neuroendocrine pathways, secondary analyses from a human laboratory study found reduced levels of leptin and insulin (Haass-Koffler et al., 2015; Haass-Koffler et al., 2016) and increased levels of cortisol and aldosterone (Haass-Koffler et al., 2019) following i.v. administration of ghrelin in heavy-drinking individuals with alcohol dependence. Of note, participants did not receive alcohol in the aforementioned study (Leggio et al., 2014).

The goal of the present study was to provide a comprehensive assessment of neuroendocrine response to a supraphysiological pharmacological challenge with exogenous ghrelin by examining the effects on appetitive/metabolic and stress-related endocrine outcomes in heavy-drinking individuals who also received i.v. alcohol under a controlled experimental setting.

Methods

Setting and Participants

Data were collected under a human laboratory study conducted at the National Institutes of Health (NIH) Clinical Center in Bethesda, Maryland. The protocol was approved by the NIH Addictions Institutional Review Board (13-AA-0043), registered at ClinicalTrials.gov (NCT01779024), and conducted under an Investigational New Drug application (IND #117,778) following review by the Food and Drug Administration. The primary goal of the parent study was to examine the effects exogenous ghrelin on i.v. alcohol self-administration and brain functional activity in heavy alcohol drinkers (Farokhnia et al., 2018). Potential candidates were first screened through a phone interview, followed by an in-person screening visit. Inclusion/exclusion criteria were assessed, and eligible individuals were enrolled after providing written, informed consent. Enrolled participants were non-treatment-seeking, heavy-drinking (>15 and >20 standard drinks per week for females and males, respectively), alcohol-dependent (DSM-IV-TR) individuals with no significant health problems. For the complete list of eligibility criteria, see supplementary Appendix 1.

Design and Procedures

A detailed description of the parent study was previously reported (Farokhnia et al., 2018). Briefly, each participant underwent up to 4 experimental sessions (Figure 1): 2 i.v. alcohol self-administration (IV-ASA) and 2 brain functional magnetic resonance imaging (fMRI) sessions. The fMRI experiment included an i.v. alcohol clamp (IV-AC) designed to achieve a target blood alcohol concentration. Alcohol administration procedures were performed in accordance with the National Institute on Alcohol Abuse and Alcoholism Council Guidelines on Alcohol Administration (https://niaaa.nih.gov/Resources/ResearchResources/job22.htm). Each experiment (IV-ASA and IV-AC) had a crossover, randomized, double-blind, placebo-controlled design during which participants received a 10-minute loading dose of i.v. acyl-ghrelin (3 mcg/kg) or placebo, followed by a continuous acyl-ghrelin (16.9 ng/kg/min) or placebo infusion, until the end of each session. For more details about the experimental compounds, see supplementary Appendix 2.

Intravenous Alcohol Self-Administration

During each 120-minute IV-ASA session, participants were given the opportunity to self-administer alcohol via the Computer-Alcohol Infusion System by pressing a button in a progressive ratio manner. Each alcohol infusion was designed to increase the breath alcohol concentration (BrAC) by 7.5 mg% over 2.5 minutes, with a subsequent decrease of 0.5 mg%/min, until the following infusion. Participants were not allowed to administer alcohol beyond a maximum BrAC of 120 mg%. Primary results of this paradigm showed that exogenous ghrelin administration, compared to placebo, significantly increased the total amount of alcohol self-administered (Farokhnia et al., 2018).

Intravenous Alcohol Clamp

A predetermined dose of i.v. alcohol was administered as part of a brain fMRI experiment. This alcohol infusion was designed to increase the BrAC linearly to 80 mg% within 20 minutes and maintain (clamp) the BrAC at this level for 15 minutes (therefore, 35 minutes in total). Primary results of this paradigm showed that exogenous ghrelin, compared to placebo, differentially modulated brain functional activity while anticipating alcohol vs food reward (Farokhnia et al., 2018).

Participants were admitted to the NIH Clinical Center the evening before each experimental session and were discharged

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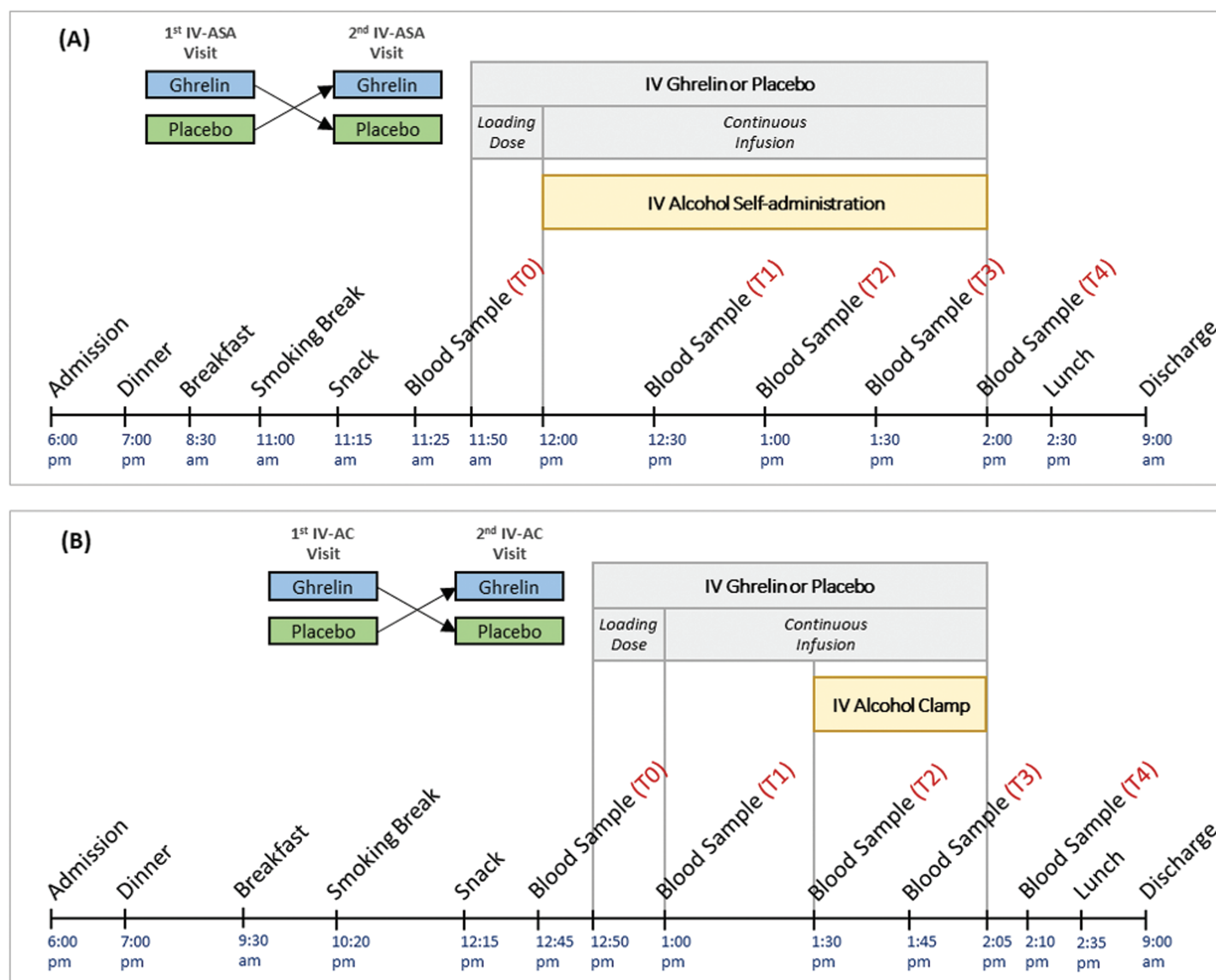

Figure 1. Schematic outline of the events and blood sampling time-points during (A) i.v. alcohol self-administration (IV-ASA) experiment (2 visits), and (B) i.v. alcohol clamp (IV-AC) experiment (2 visits). Each participant underwent up to 4 experimental sessions. During each session, a 10-minute loading dose of i.v. acyl-ghrelin (3 mcg/kg) or placebo was administered, followed by a continuous infusion of acyl-ghrelin (16.9 ng/kg/min) or placebo until the end of the session.

the morning after; therefore, each experimental session was conducted under a controlled inpatient setting, where parameters such as alcohol intake, smoking breaks, and diet were closely monitored and standardized. Three standardized meals and 1 standardized snack were served during each visit. A washout period of at least 3 days was applied between study visits. For additional details, see Figure 1 and supplementary Appendix 3.

Blood Collection, Processing, and Assays

Repeated blood samples (5 time-points; see Figure 1) were obtained during each experimental visit via a saline lock i.v. catheter that was fixed in the antecubital fossa of the nondominant arm. Blood concentrations of the following hormones were measured: total ghrelin, acyl-ghrelin, GH, leptin, GLP-1, pancreatic polypeptide (PP), gastric inhibitory peptide, insulin, insulin-like growth factor-1 (IGF-1), cortisol, prolactin, and aldosterone. Details regarding blood collection, processing, and assays are presented in supplementary Appendix 4. Values below the lower limit of quantitation (LLOQ) for each assay were set to 1/2 of the LLOQ (Keizer et al., 2015).

Statistical Methods

Demographic characteristics of the study sample were summarized with descriptive statistics (mean and standard error for continuous variables, number and percent for categorical variables). Data from the ghrelin sessions of the IV-ASA and IV-AC were used to characterize kinetic parameters of peripheral acyl-ghrelin and total ghrelin concentrations in this study. Noncompartmental analyses were run, and the linear trapezoidal rule was applied for estimations (Phoenix WinNonlin, Pharsight Corp., version 6.3; Mountain View, CA). Calculated parameters included area under the plasma concentration-time curve ($AUC_{0\text{-last}}$), maximum plasma concentration (C_{max}), time of C_{max} (T_{max}), half-life, mean residence time (MRT), clearance (Cl), and volume of distribution (Vd). Baseline correction was performed using pre-dose concentrations of the placebo session. This correction was done to minimize the within-day variability of each hormone and to provide a more accurate estimation of acyl-ghrelin and total ghrelin kinetic parameters (Scheff et al., 2011). Neuroendocrine outcomes were first tested for statistical outliers and normal distribution. Leptin, GLP-1, and GH data for the IV-ASA experiment and acyl-ghrelin and total ghrelin data for the IV-AC experiment were

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not normally distributed and therefore were log10 transformed, which led to significant improvement in normality. Baseline (T0) concentrations of the endocrine outcomes were compared between the 2 sessions (i.v. ghrelin vs placebo) of each experiment using independent samples *t* test. To examine i.v. ghrelin's acute effect, we first compared the change (Δ) in blood concentrations of each neuroendocrine outcome from baseline (T0) to post-drug (T1) in response to i.v. ghrelin vs placebo using independent samples *t* test. Next, we examined the pattern/time course of these hormones over a longer timeframe, that is, during the i.v. ghrelin vs placebo sessions. For this purpose, repeated measurements of each neuroendocrine outcome were analyzed via linear mixed-effects models with drug condition (i.v. ghrelin, placebo), blood sampling time-point (T1, T2, T3, T4), and drug × time-point interaction as fixed effects, individual participants as a random effect, and blood concentrations of each hormone as the outcome. Age, gender, body weight, session order (i.v. ghrelin or placebo first), total number of alcohol infusions self-administered (IV-ASA experiment only), and baseline (T0) concentration of each hormone (first time-point) were also included as covariates. Partial eta squared (η^2_p) values were also calculated to indicate effect sizes. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) software (IBM Corp., version 25; Armonk, NY), and significance level was set at $P<.05$ (2-tailed).

Results

Sample Characteristics and Ghrelin Concentrations

Of 77 individuals screened, 18 signed the informed consent and were enrolled. A final sample of 11 and 8 participants completed both IV-ASA sessions and both IV-AC sessions, respectively, and were included in the analyses (supplementary Figure 1). Table 1 summarizes the demographic characteristics of the study sample. Enrolled participants were predominantly male and African American. Although not a required eligibility criterion, all participants had a current diagnosis of alcohol dependence based on DSM-IV-TR.

As expected, i.v. ghrelin administration significantly increased blood concentrations of acyl-ghrelin, total ghrelin, and GH (see supplementary Figures 2 and 3), confirming that the supraphysiological challenge with exogenous acyl-ghrelin was successful (i.e., ghrelin levels increased) and pharmacologically effective (i.e., GH levels increased). Ten individuals from the IV-ASA experiment and 6 individuals from the IV-AC experiment had complete data for calculation of acyl-ghrelin and total ghrelin kinetic parameters (supplementary Tables 1 and 2). Respective spaghetti plots are also depicted in supplementary Figures 4 and 5.

Neuroendocrine Outcomes

Intravenous Alcohol Self-Administration Experiment

Figure 2 outlines the IV-ASA experiment neuroendocrine outcomes.

Baseline (T0) concentrations of the measured hormones did not significantly differ between the 2 IV-ASA sessions, except for significantly higher baseline insulin levels prior to i.v. ghrelin than placebo administration (supplementary Table 3).

Comparison of change (Δ) from T0 to T1 showed that IV ghrelin, compared to placebo, significantly increased blood concentrations of GLP-1 (t [20] = −4.91, $P<.001$), PP (t [20] = −5.27, $P<.001$), cortisol (t [19] = −4.65, $P<.001$), and prolactin (t [18] = −4.56, $P<.001$) (Figure 2; Table 2).

Analysis of repeated measures during the IV-ASA experiment (T1, T2, T3, and T4) after controlling for baseline (T0) found lower leptin (F [1, 57.78] = 6.28, $P=.01$) and higher GLP-1 (F [1, 55.28] = 75.73, $P<.001$), PP (F [1, 61.11] = 55.56, $P<.001$), insulin (F [1, 50.75] = 21.85, $P<.001$), cortisol (F [1, 63.34] = 186.75, $P<.001$), prolactin (F [1, 59.93] = 87.45, $P<.001$), and aldosterone (F [1, 65.63] = 15.62, $P<.001$) concentrations under i.v. ghrelin compared to placebo, as indicated by significant drug main effects. Significant drug × time-point interaction effects were also found for PP (F [3, 61.65] = 5.71, $P=.002$) and prolactin (F [3, 53.39] = 5.65, $P=.002$) (Figure 2; Table 3). Of note, the significant drug main effect on insulin appears to be a carryover effect of significantly higher levels of insulin at baseline, that is, prior to ghrelin vs placebo administration (Figure 2E).

Intravenous Alcohol Clamp Experiment

Figure 3 outlines the IV-AC experiment neuroendocrine outcomes. Baseline (T0) concentrations of the measured hormones did not significantly differ between the 2 IV-AC sessions (supplementary Table 4).

Comparison of change (Δ) from T0 to T1 showed that i.v. ghrelin, compared to placebo, significantly increased blood concentrations GLP-1 (t [11] = −4.67, $P=.001$), cortisol (t [11] = −4.76, $P=.001$), and prolactin (t [10] = −4.96, $P=.001$); a trend-level increase in PP (t [10] = −1.85, $P=.06$) was also found (Figure 3; Table 4).

Table 1. Demographic Characteristics of the Study Sample

	IV-ASA experiment (n = 11)	IV-AC experiment (n = 8)
Age, years, M (SEM)	39.86 (3.54)	42.50 (3.12)
Gender, males, n (%)	8 (72.72)	6 (75)
Race, African Americans, n (%)	9 (81.81)	7 (87.5)
Education, years, M (SEM)	13.36 (0.49)	13.75 (0.70)
Annual income[a], n (%)		
Below average	7 (63.63)	5 (62.5)
Average	3 (27.27)	2 (25)
Above average	1 (9.09)	1 (12.5)
Body weight, kg, M (SEM)	77.44 (3.29)	77.90 (3.50)
BMI, kg/m^2, M (SEM)	25.42 (0.87)	25.88 (0.98)
Family history density of problem drinking[b], M (SEM)	0.10 (0.03)	0.14 (0.05)
Current cigarette smoker, n (%)	8 (72.72)	5 (62.5)

Abbreviations: BMI, body mass index; IV-AC, intravenous alcohol clamp; IV-ASA, intravenous alcohol self-administration; M, mean; SEM, standard error of the mean.
*a*Below average: <$30 000; average: $30 000-$49 000; above average: ≥$50 000.
*b*Based on Family Tree Questionnaire: density of relatives (siblings, parents, grandparents) with definite problem drinking (self-reported).

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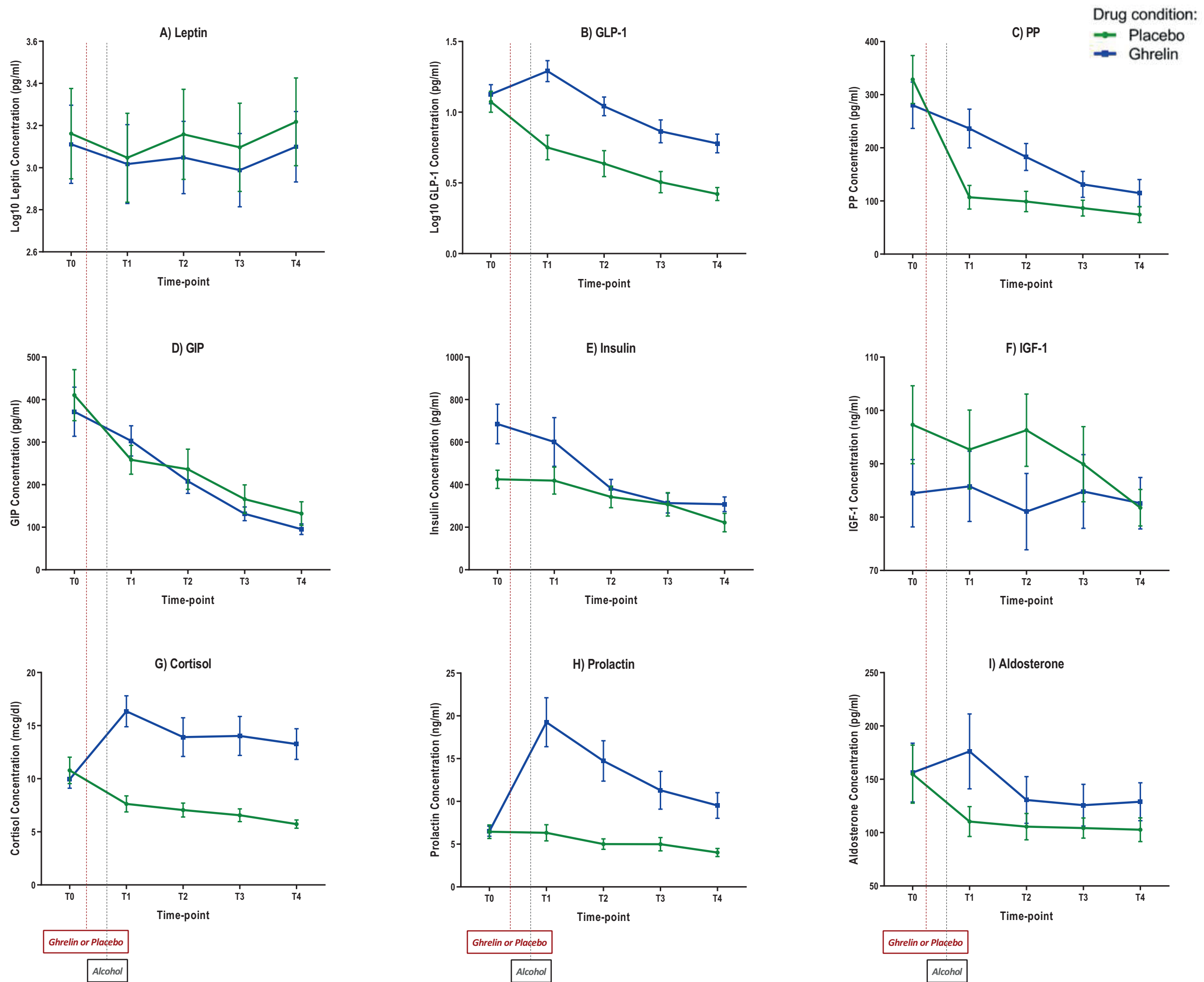

Figure 2. Blood concentrations of neuroendocrine outcomes during ghrelin and placebo sessions of the i.v. alcohol self-administration (IV-ASA) experiment. A 10-minute loading dose of i.v. acyl-ghrelin (3 mcg/kg) or placebo was administered, followed by a continuous infusion of acyl-ghrelin (16.9 ng/kg/min) or placebo until the end of each session. Alcohol self-administration started simultaneously with the ghrelin/placebo continuous infusion (between T0 and T1) and continued until the end of the session (T4). T0: 25 minutes before the start of the ghrelin/placebo loading dose; T1: 30 minutes after the start of the ghrelin/placebo continuous infusion; T2: 60 minutes after the start of the ghrelin/placebo continuous infusion; T3: 90 minutes after the start of the ghrelin/placebo continuous infusion; T4: 120 minutes after the start of the ghrelin/placebo continuous infusion. Blood concentrations of each hormone per time-point are expressed as mean (M) and standard error of the mean (SEM). For statistical results, see Table 2. Abbreviations: GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; IGF-1, insulin-like growth factor-1; PP, pancreatic polypeptide.

Analysis of repeated measures during the IV-ASA experiment (T1, T2, T3, and T4) after controlling for baseline (T0) found lower IGF-1 (F [1, 19.35] = 5.29, P = .03) and higher GLP-1 (F [1, 31.53] = 30.40, P < .001), PP (F [1, 36.00] = 17.47, P < .001), cortisol (F [1, 22.66] = 42.53, P < .001), prolactin (F [1, 33.37] = 134.14, P < .001), and aldosterone (F [1, 30.15] = 36.44, P < .001) concentrations under i.v. ghrelin, compared to placebo, as indicated by significant drug main effects. A significant drug × time-point interaction effect was also found for cortisol (F [3, 33.55] = 3.34, P = .03) (Figure 3; Table 5). Of note, the significant drug main effect on IGF-1 appears to be driven by an unexpected increase in IGF-1 concentrations at T3 and T4 under the placebo condition (Figure 3F).

Discussion

The present study investigated the effects of a supraphysiological challenge with exogenous ghrelin on peripheral concentrations of a range of hormones in heavy-drinking, alcohol-dependent individuals concurrently receiving i.v. alcohol. Despite methodological differences in terms of alcohol dosage, blood sampling time-points, etc., the results were in overall agreement and internally replicated across the 2 alcohol administration paradigms (i.e., IV-ASA and IV-AC). i.v. ghrelin, compared to placebo, significantly increased blood concentrations of GLP-1, PP, cortisol, and prolactin both acutely (Δ T0-T1) and during the session (T1, T2, T3, and T4, while controlling for T0). Lower levels of leptin and higher levels of aldosterone were also found during the ghrelin vs placebo session (Figure 4).

In addition to the supraphysiological challenge with exogenous ghrelin, the experiments in this study included i.v. administration of alcohol using a well-established computer-based method that limited the variability in alcohol levels/exposure typically observed after oral alcohol consumption (Cyders et al., 2020). The 2 experimental paradigms (IV-ASA and IV-AC) could be considered complementary, as they included different levels and durations of exposure to alcohol as well as different blood

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Table 2. Comparison of Change in Blood Concentrations of Neuroendocrine Outcomes, From Baseline to Post-Drug Time-Point[a], Between Placebo and Ghrelin Sessions of the IV-ASA Experiment

	Placebo session, M (SEM)	Ghrelin session, M (SEM)	Independent samples *t* test
Δ Leptin (Log10) concentration (pg/mL)	−0.11 (0.02)	−0.09 (0.02)	t (18) = −0.62, P = .54
Δ GLP-1 (Log10) concentration (pg/mL)	−0.32 (0.04)	0.16 (0.08)	t (20) = −4.91, P < .001
Δ PP concentration (pg/mL)	−221.46 (28.44)	−43.87 (18.01)	t (20) = −5.27, P < .001
Δ GIP concentration (pg/mL)	−151.94 (51.00)	−68.62 (79.23)	t (20) = −0.88, P = .38
Δ Insulin concentration (pg/mL)	−81.95 (39.32)	−84.42 (144.79)	t (18) = 0.01, P = .98
Δ IGF-1 concentration (ng/mL)	−4.63 (4.94)	1.28 (4.19)	t (20) = −0.91, P = .37
Δ Cortisol concentration (mcg/dL)	−3.15 (1.14)	6.24 (1.70)	t (19) = −4.65, P < .001
Δ Prolactin concentration (ng/mL)	−0.13 (0.78)	12.74 (2.70)	t (18) = −4.56, P < .001
Δ Aldosterone concentration (pg/mL)	−44.41 (18.31)	18.07 (33.49)	t (19) = −1.67, P = .11

Abbreviations: GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; IGF-1, insulin-like growth factor-1; IV-ASA, i.v. alcohol self-administration; M, mean; PP, pancreatic polypeptide; SEM, standard error of the mean.
*a*Baseline (T0): 25 minutes before the start of the ghrelin/placebo loading dose, post-drug (T1): 30 minutes after the start of the ghrelin/placebo continuous infusion. See Figure 1 for more details.

Table 3. Drug, Time-Point, and Drug × Time-Point Effects on Neuroendocrine Outcomes During the IV-ASA Experiment

	Drug[a] main effect	Time-point[b] main effect	Drug × time-point interaction effect
Leptin	F (1, 57.78) = 6.28, P = .01 η^2_p = 0.09	F (3, 55.38) = 5.26, P = .003 η^2_p = 0.22	F (3, 55.38) = 0.03, P = .99 η^2_p = 0.002
GLP-1	F (1, 55.28) = 75.73, P < .001 η^2_p = 0.57	F (3, 59.24) = 20.43, P < .001 η^2_p = 0.50	F (3, 59.38) = 1.75, P = .16 η^2_p = 0.08
PP	F (1, 61.11) = 55.56, P < .001 η^2_p = 0.47	F (3, 61.65) = 14.45, P < .001 η^2_p = 0.41	F (3, 61.65) = 5.71, P = .002 η^2_p = 0.21
GIP	F (1, 66.97) = 3.66, P = .06 η^2_p = 0.05	F (3, 60.30) = 25.61, P < .001 η^2_p = 0.56	F (3, 60.30) = 1.63, P = .19 η^2_p = 0.07
Insulin	F (1, 50.75) = 21.85, P < .001 η^2_p = 0.30	F (3, 46.58) = 12.36, P < .001 η^2_p = 0.44	F (3, 46.58) = 2.15, P = .10 η^2_p = 0.12
IGF-1	F (1, 60.68) = 0.11, P = .73 η^2_p = 0.001	F (3, 60.18) = 0.29, P = .82 η^2_p = 0.01	F (3, 60.21) = 1.05, P = .37 η^2_p = 0.05
Cortisol	F (1, 63.34) = 186.75, P < .001 η^2_p = 0.74	F (3, 57.40) = 3.60, P = .01 η^2_p = 0.15	F (3, 57.40) = 0.70, P = .55 η^2_p = 0.03
Prolactin	F (1, 59.93) = 87.45, P < .001 η^2_p = 0.59	F (3, 53.40) = 15.06, P < .001 η^2_p = 0.45	F (3, 53.39) = 5.65, P = .002 η^2_p = 0.24
Aldosterone	F (1, 65.63) = 15.62, P < .001 η^2_p = 0.19	F (3, 59.20) = 2.61, P = .05 η^2_p = 0.11	F (3, 59.20) = 1.73, P = .17 η^2_p = 0.08

Abbreviations: GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; IGF-1, insulin-like growth factor-1; IV-ASA, i.v. alcohol self-administration; PP, pancreatic polypeptide.
*a*Intravenous ghrelin or placebo.
*b*T1 (30 minutes after the start of the ghrelin/placebo continuous infusion), T2 (60 minutes after the start of the ghrelin/placebo continuous infusion), T3 (90 minutes after the start of the ghrelin/placebo continuous infusion), and T4 (120 minutes after the start of the ghrelin/placebo continuous infusion). T0 (baseline) was included as a covariate in each model, along with age, gender, body weight, session order, and total number of alcohol infusions self-administered. See Figure 1 for more details.

sampling time-points in relation to both i.v. ghrelin and alcohol administration (see Figure 1). Specifically, the IV-ASA experiment had a longer duration, included a variable dose of alcohol (proportionate to the amount that each participant decided to self-administer), and all sampling time-points occurred under i.v. ghrelin (or i.v. placebo) plus alcohol. On the other hand, the IV-AC had a shorter duration, included a predetermined dose of alcohol (designed to achieve a target blood alcohol concentration), and had 1 sampling time-point (T1) that occurred under only i.v. ghrelin (or i.v. placebo) infusion, before alcohol administration began, hence providing an opportunity to relatively tease out the effects of "ghrelin" per se vs "ghrelin plus alcohol." Nonetheless, our observations are consistent with previous work on endocrine response to exogenous ghrelin administration (for review, see Garin et al., 2013b) and provide novel information among a clinically relevant population (i.e., heavy-drinking individuals with alcohol dependence) and in the context of alcohol use, which is also a major modulator of peripheral and central endocrine pathways.

A large body of preclinical and clinical evidence indicates that peripheral leptin levels are associated with biobehavioral correlates of alcohol craving, use, and withdrawal (Bach et al., 2020). Results of the present study are comparable with our previous finding of exogenous ghrelin-induced reduction in circulating leptin levels in heavy-drinking individuals with alcohol dependence in a cue-reactivity study without actual alcohol administration (Haass-Koffler et al., 2015). Ghrelin and leptin have inverse functions in relation to appetite, food intake, metabolism, and alcohol use, and it has been suggested that leptin negatively mediates peripheral ghrelin levels (Klok et al., 2007; Nogueiras et al., 2008). It is important to note that although leptin levels were lower under ghrelin, compared to

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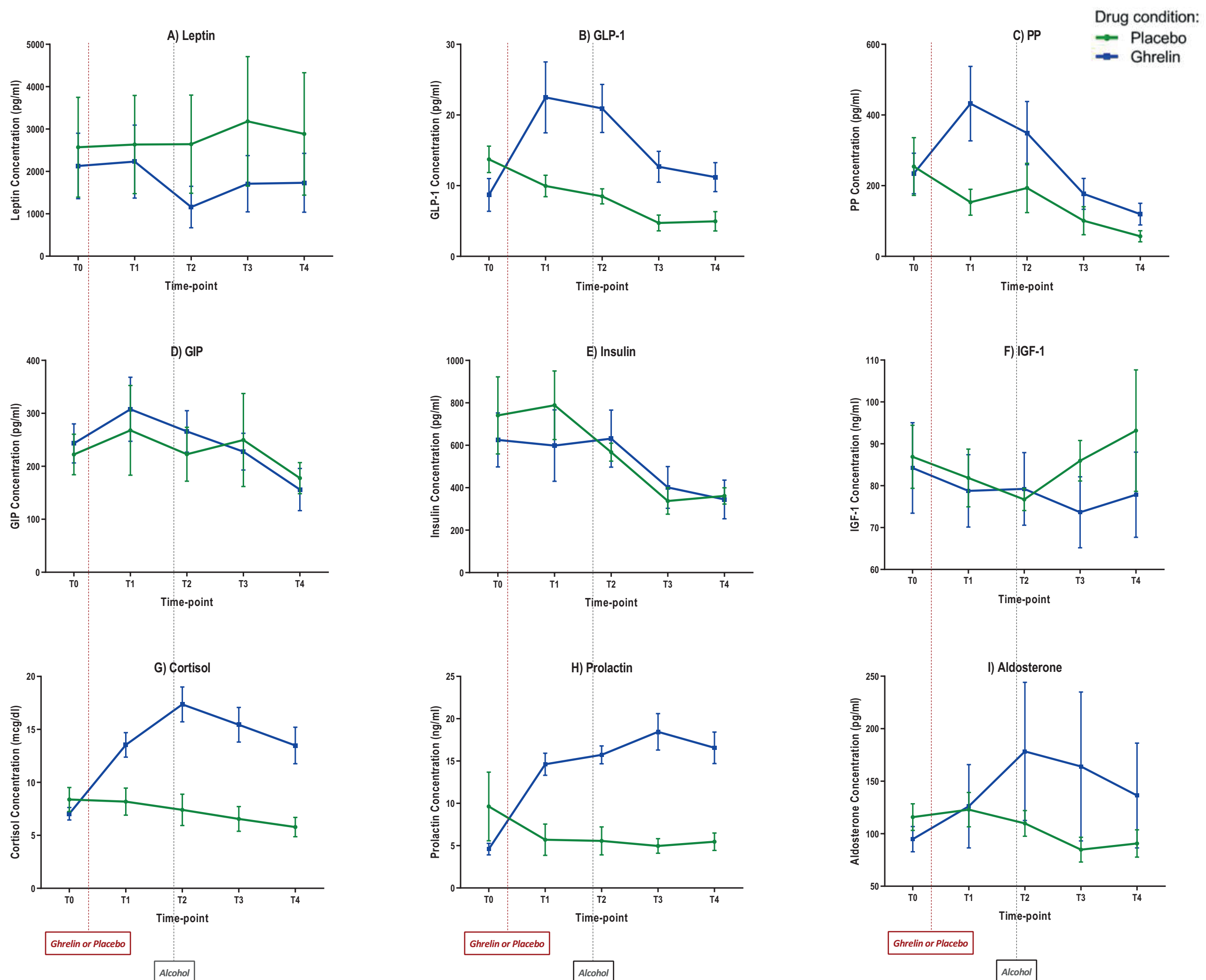

Figure 3. Blood concentrations of neuroendocrine outcomes during ghrelin and placebo sessions of the i.v. alcohol clamp (IV-AC) experiment. A 10-minute loading dose of i.v. acyl-ghrelin (3 mcg/kg) or placebo was administered, followed by a continuous infusion of acyl-ghrelin (16.9 ng/kg/min) or placebo until the end of each session. Alcohol clamp started at T2 and continued until the end of the session (5 minutes before T4). T0: 5 minutes before the start of the ghrelin/placebo loading dose; T1: at the end of the ghrelin/placebo loading dose and the start of the ghrelin/placebo continuous infusion; T2: 30 minutes after the start of the ghrelin/placebo continuous infusion; T3: 45 minutes after the start of the ghrelin/placebo continuous infusion; T4: 70 minutes after the start of the ghrelin/placebo continuous infusion. Blood concentrations of each hormone per time-point are expressed as mean (M) and standard error of the mean (SEM). For statistical results, see Table 3. Abbreviations: GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; IGF-1, insulin-like growth factor-1; PP, pancreatic polypeptide.

placebo, during both IV-ASA and IV-AC experiments (Figures 2A and 3A), the difference did not reach statistical significance for the IV-AC experiment. In general, due to inherent limitations of the present study (e.g., small sample size, inter-individual variability, secondary nature of the analyses), we encourage the readers to focus more on the pattern and direction of hormonal changes (depicted in Figures 2–4) rather than pure statistical results (presented in Tables 2–5). This approach is consistent with the overall goal of the present study, that is, to evaluate how these hormones "behave" in response to and under i.v. ghrelin, compared to placebo, in the context of alcohol use.

Akin to the data presented here (Figures 2B and 3B), studies in both rodents and humans have observed an increase in GLP-1 concentrations following ghrelin administration (Tong et al., 2016; Lindqvist et al., 2017). GLP-1 is an incretin produced mainly by L-cells of the intestinal mucosa and plays a key role in regulating food intake and glucose homeostasis (Müller et al., 2019). While some studies suggest that ghrelin's effect on GLP-1 production and secretion might be driven by direct actions on intestinal L-cells, others do not confirm such a direct interaction (Jepsen et al., 2019). It appears that the cross-talk between insulinostatic ghrelin and insulinotropic GLP-1 is largely mediated through indirect glucose-dependent mechanisms (Djurhuus et al., 2002; Damdindorj et al., 2012; Page et al., 2018), although the exact molecular mechanism remains unknown. Preclinical studies have suggested that ghrelin and GLP-1 signaling may overlap in the nucleus accumbens to regulate alcohol intake and reward (Abtahi et al., 2018). Therefore, targeting these endocrine pathways offers potential novel treatment options for individuals with AUD (Farokhnia et al., 2019b; Jerlhag, 2020). In a previous cue-reactivity study with i.v. ghrelin, we found different results for insulin and GLP-1. Specifically, i.v. ghrelin reduced blood insulin levels without significantly affecting GLP-1 levels (Haass-Koffler et al., 2016). Together, these findings suggest that unlike other hormones investigated here (e.g., leptin, cortisol, and aldosterone, for which we did replicate

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Table 4. Comparison of Change in Blood Concentrations of Neuroendocrine Outcomes, From Baseline to Post-Drug Time-Point[a], Between Placebo and Ghrelin Sessions of the IV-AC Experiment

	Placebo session, M (SEM)	Ghrelin session, M (SEM)	Independent samples *t* Test
Δ Leptin Concentration (pg/mL)	65.79 (145.13)	102.95 (175.28)	t (11) = –0.16, P = 87
Δ GLP-1 Concentration (pg/mL)	–3.77 (1.64)	13.81 (3.16)	t (11) = –4.67, P = 001
Δ PP Concentration (pg/mL)	–36.07 (30.36)	197.86 (102.96)	t (10) = –1.85, P = 06
Δ GIP Concentration (pg/mL)	45.58 (47.83)	64.36 (38.40)	t (11) = –0.31, P = 76
Δ Insulin Concentration (pg/mL)	47.67 (57.69)	–26.45 (87.58)	t (11) = 0.68, P = 51
Δ IGF-1 Concentration (ng/mL)	–5.06 (3.77)	–5.47 (8.43)	t (11) = 0.04, P = 96
Δ Cortisol Concentration (mcg/dL)	–0.26 (0.72)	6.91 (1.24)	t (11) = –4.76, P = 001
Δ Prolactin Concentration (ng/mL)	–0.23 (0.24)	9.73 (1.99)	t (10) = –4.96, P = 001
Δ Aldosterone Concentration (pg/mL)	2.43 (6.97)	35.51 (30.35)	t (9) = –0.96, P = 35

Abbreviations: GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; IGF-1, insulin-like growth factor-1; IV-AC, i.v. alcohol clamp; M, mean; PP, pancreatic polypeptide; SEM, standard error of the mean.
[a]Baseline (T0): 5 minutes before the start of the ghrelin/placebo loading dose, post-drug (T1): at the end of the ghrelin/placebo loading dose and the start of the ghrelin/placebo continuous infusion. See Figure 1 for more details.

Table 5. Drug, Time-Point, and Drug × Time-Point Effects on Neuroendocrine Outcomes During the IV-AC Experiment

	Drug[a] main effect	Time-point[b] main effect	Drug × time-point interaction effect
Leptin	F (1, 8.58) = 0.19, P = .67 $\eta^2_p = 0.02$	F (3, 35.04) = 0.44, P = .72 $\eta^2_p = 0.03$	F (3, 35.06) = 0.97, P = .41 $\eta^2_p = 0.07$
GLP-1	F (1, 31.53) = 30.40, P < .001 $\eta^2_p = 0.49$	F (3, 35.07) = 6.36, P = .001 $\eta^2_p = 0.35$	F (3, 35.07) = 0.85, P = .47 $\eta^2_p = 0.06$
PP	F (1, 36.00) = 17.47, P < .001 $\eta^2_p = 0.32$	F (3, 36.00) = 7.42, P = .001 $\eta^2_p = 0.38$	F (3. 36.00) = 1.59, P = .20 $\eta^2_p = 0.11$
GIP	F (1, 37.00) = 0.01, P = .90 $\eta^2_p = 0.0004$	F (3, 37.00) = 2.44, P = .08 $\eta^2_p = 0.16$	F (3, 37.00) = 0.57, P = .63 $\eta^2_p = 0.04$
Insulin	F (1, 19.75) = 0.67, P = .42 $\eta^2_p = 0.03$	F (3, 31.45) = 13.36, P < .001 $\eta^2_p = 0.56$	F (3, 31.45) = 0.41, P = .74 $\eta^2_p = 0.03$
IGF-1	F (1, 19.35) = 5.29, P = .03 $\eta^2_p = 0.21$	F (3, 34.24) = 0.31, P = .81 $\eta^2_p = 0.02$	F (3, 34.24) = 0.88, P = .46 $\eta^2_p = 0.07$
Cortisol	F (1, 22.66) = 42.53, P < .001 $\eta^2_p = 0.65$	F (3, 33.55) = 4.32, P = .01 $\eta^2_p = 0.27$	F (3, 33.55) = 3.34, P = .03 $\eta^2_p = 0.23$
Prolactin	F (1, 33.37) = 134.14, P < .001 $\eta^2_p = 0.80$	F (3, 32.66) = 1.02, P = 0.39 $\eta^2_p = 0.08$	F (3, 32.62) = 0.50, P = .68 $\eta^2_p = 0.04$
Aldosterone	F (1, 30.15) = 36.44, P < .001 $\eta^2_p = 0.54$	F (3, 28.88) = 0.68, P = .56 $\eta^2_p = 0.06$	F (3, 28.88) = 1.20, P = .32 $\eta^2_p = 0.11$

Abbreviations: GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; IGF-1, insulin-like growth factor-1; IV-AC, i.v. alcohol clamp; PP, pancreatic polypeptide.
[a]Intravenous ghrelin and placebo
[b]T1 (at the end of the ghrelin/placebo loading dose and the start of the ghrelin/placebo continuous infusion), T2 (30 minutes after the start of the ghrelin/placebo continuous infusion), T3 (45 minutes after the start of the ghrelin/placebo continuous infusion), and T4 (70 minutes after the start of the ghrelin/placebo continuous infusion). T0 (baseline) was included as a covariate in each model, along with age, gender, body weight, and session order. See Figure 1 for more details.

our previous findings), the crosstalk between ghrelin and hormones such as insulin and GLP-1 may be more subject to variability and sensitive to different experimental conditions and settings. For example, in the previous study, there was no alcohol coadministration, fasting conditions differed, and the overall design was quite different.

Exogenous ghrelin administration, compared to placebo, had a robust effect on PP levels in this study. PP is a 36-amino acid polypeptide produced by PP cells of pancreatic islets (islets of Langerhans). PP is involved in self-regulation of endocrine and exocrine functions of the pancreas and acts primarily as an anorexigenic hormone by suppressing food intake, delaying gastric emptying, and increasing energy expenditure (Lonovics et al., 1981). PP has been suggested to be a biomarker of vagal activity (Schwartz, 1983b). While alcohol has been shown to decrease PP (Sehested et al., 1998), ghrelin has been found to increase PP levels (Arosio et al., 2003). In this study, it is likely that i.v. ghrelin administration initially increased serum PP levels, but this stimulatory effect was blunted by administration of alcohol. This interpretation is supported by comparing the PP results during the IV-ASA vs IV-AC experiments, as the variation in timing of i.v. ghrelin and alcohol administration between the studies shows a distinct stimulatory effect of ghrelin and inhibitory effect of alcohol on PP. In other words, the interval between T0 and T1 of the IV-AC experiment, when only i.v. ghrelin (and no i.v. alcohol) is on board, revealed a clear separation where i.v. ghrelin increased PP concentrations (Figure 3C). Consistent with this observation, during the IV-ASA experiment, which did not include an alcohol-free blood sampling time-point, exogenous ghrelin administration, compared to placebo, clearly blunted the alcohol-induced reduction in PP concentrations, which even resulted in a significant drug × time-point interaction (Figure 2C). Given that the secretion of PP is tightly regulated by vagal cholinergic mechanisms (Schwartz, 1983a) and ghrelin receptors are widely expressed in the vagal afferent neurons (Date, 2012), changes in PP concentrations in response to exogenous

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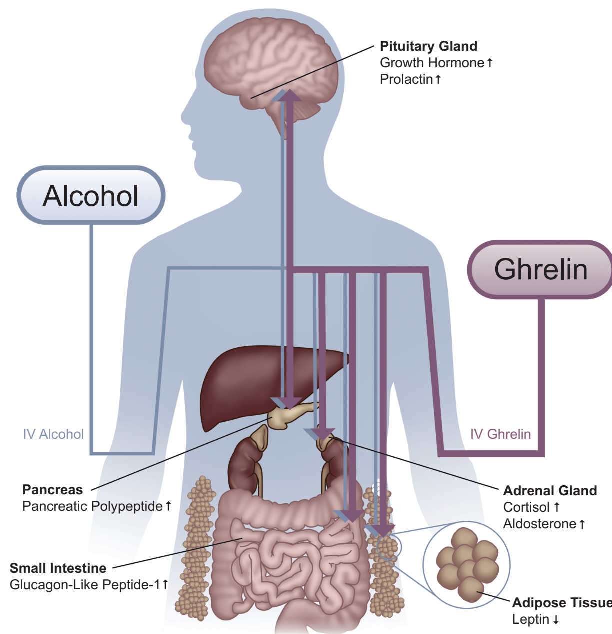

Figure 4. A graphical summary of the main hormonal changes, found in this study, in response to i.v. ghrelin, compared to placebo. Findings were in overall agreement across the 2 alcohol administration paradigms (i.e., IV-ASA and IV-AC). Increased levels of growth hormone, prolactin, cortisol, aldosterone, pancreatic polypeptide, and glucagon-like peptide-1, and decreased levels of leptin were observed under i.v. ghrelin administration, compared to the placebo condition. Abbreviations: AC, alcohol clamp; ASA, alcohol self-administration.

ghrelin administration can be interpreted as a proxy of GHS-R1a activity on vagal dendrites in the periphery. Accordingly, peripheral activation of vagal GHS-R1a has been proposed as a main route carrying the ghrelin signal to the CNS (Dockray, 2013; Date, 2014; Tamboli et al., 2017). Of note, we previously reported that exogenous ghrelin administration significantly reduced systolic and diastolic blood pressure in this (Farokhnia et al., 2018) and previous (Leggio et al., 2014) work, which is consistent with other reports on GHSR1a-dependent autonomic activity of ghrelin (Garin et al., 2013a; Zhang et al., 2017).

Consistent with our findings, several preclinical and clinical studies have found that ghrelin stimulates the production and/or secretion of cortisol, aldosterone, and prolactin, leading to increased concentrations of these hormones in the periphery (Arvat et al., 2001; Vestergaard et al., 2007; Messini et al., 2011; Zhang et al., 2017; Haass-Koffler et al., 2019; Akalu et al., 2020). Ghrelin-knockout models in rodents have identified the centrally projecting Edinger-Wesphal nucleus, specifically urocortin 1 neurons, as a link between GHS-R1a activation and corticosterone response (Spencer et al., 2012). Furthermore, GHS-R1a is expressed throughout the CNS, most notably in the hypothalamus pituitary unit, and ghrelin has been found to increase corticotropin-releasing factor mRNA in hypothalamic 4b cells in vitro (Gnanapavan et al., 2002; Shuto et al., 2002; Kageyama et al., 2012). The higher concentrations of aldosterone under i.v. ghrelin, compared to placebo, observed in this study (Figures 2I and 3I) are consistent with previous clinical (Zhang et al., 2017; Haass-Koffler et al., 2019) and preclinical (Andreis et al., 2003; Milosević et al., 2010; Rucinski et al., 2012) reports that ghrelin-induced activation of the HPA axis extends to both glucocorticoids and mineralocorticoids and that manipulation of the ghrelin system has a global stimulatory effect on the adrenal cortex. The precise mechanism of ghrelin-induced increases in prolactin levels remains unclear, although some evidence suggests a mechanistic pairing of GHS-R1a activity and prolactin secretion (Rubinfeld et al., 2004). Together, the established GH response, adrenocorticotropic hormone secretion, and evidence of prolactin release point to ghrelin as a strong pituitary releasing agent. Of note, prolactin has been suggested to be a neuromodulator of extrahypothalamic dopaminergic activity (Hernández et al., 1994), and alcohol has also been found to modulate peripheral prolactin levels (Frias et al., 2000).

The present study had several strengths and limitations. The sample had a relatively small size and was limited to heavy-drinking individuals with alcohol dependence. The strict inclusionary and exclusionary criteria for enrollment (supplementary Appendix 1) resulted in a homogenous sample of participants, thus reducing random variability in our measures and analyses. However, this factor limits the generalizability of our findings. As a human laboratory study, the experimental settings (e.g., drug dosage, meals, blood sampling time-points) were strictly controlled before and during each experiment. Although such a design provides a rigorous research platform, it may not reflect a real-world condition and the results may not be generalizable to other settings. Two different, yet complementary, i.v. alcohol administration paradigms (IV-ASA and IV-AC) were employed, and the results were internally replicated with some statistical differences across the 2 paradigms. Application of i.v. alcohol minimizes variation in alcohol pharmacokinetics and is therefore suitable for such studies, but this route bypasses the gastrointestinal tract and the hepatic first pass metabolism, factors that may influence at least some of the neuroendocrine outcomes investigated here. Given the secondary nature of this study, inter-individual variability, and small sample size, we were not able to analyze possible clinical/behavioral correlates of the observed neuroendocrine effects in response to exogenous ghrelin administration—a relevant question that should be investigated in future studies with a larger sample and an a priori design for this purpose. Another limitation is that glucose levels were not measured during the experiments. Given that ghrelin plays an important role in glucose regulation, whether the effects of exogenous ghrelin on glucose-regulating hormones (e.g., GLP-1) are dependent on, or independent of, glucose levels remains unanswered. Finally, we had a placebo session to be compared to the i.v. ghrelin condition, but i.v. alcohol was not matched with a control and participants received alcohol during all sessions, because of the design of the parent study. The effects of the supraphysiological challenge with ghrelin on neuroendocrine outcomes appeared to surpass the effects of alcohol, at least with the doses used in this study. That said, disentangling the effects of ghrelin vs alcohol would require a fully factorial 2 (i.v. ghrelin vs placebo) × 2 (i.v. alcohol vs control) design.

In conclusion, the findings of the present study, gathered from a clinically relevant sample of heavy-drinking individuals with alcohol dependence, provide a deeper insight into the complex interplay between ghrelin and appetitive, metabolic, and stress-related endocrine pathways in the context of alcohol use. More studies are required to understand the mechanisms underlying these effects and their potential direct and/or indirect relevance to alcohol-related behaviors.

Supplementary Materials

Supplementary data are available at *International Journal of Neuropsychopharmacology (IJNPPY)* online.

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Statement of Interest

The authors report no biomedical financial interests or potential conflicts of interest. Dr Leggio is a federal employee; outside his federal work, he receives royalties from Rutledge for a textbook and an honorarium from the UK Medical Council on Alcohol for serving as editor-in-chief of Alcohol and Alcoholism.

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