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Changes in circulating peptide YY and ghrelin are associated with early smoking relapse

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ABSTRACT

Ghrelin and peptide YY (PYY) during ad libitum smoking have been associated with decreased reported craving (ghrelin) and increased positive affect (PYY), and higher baseline ghrelin levels predicted subsequent increased risk of smoking relapse. The current study assessed PYY and ghrelin during ad libitum smoking and again after the initial 48 h of a smoking cessation attempt. The data compared smokers who abstained for 28 days (n = 37), smokers who relapsed (n = 54), and nonsmokers (n = 37). Plasma samples and subjective measures assessing craving and mood were collected at the beginning of each session. Results showed that relapsers experienced greater levels of distress (ps < 0.01). While nonsmokers and abstainers showed no change in ghrelin across the initial 48 h, relapsers declined ($p < 0.01$). With PYY, relapsers increased ($p < 0.05$) across the early abstinent phase. PYY and ghrelin may be useful predictors of relapse, specifically in reference to early withdrawal.

1. Introduction

The link between appetite regulation and addiction reward has been established in preclinical and clinical studies (for review see: Abizaid, 2009; Dickson et al., 2011; Engel & Jerlhag, 2014; Hillemacher et al., 2007). Recent evidence indicates a role for appetite hormones in craving, relapse, and the reward properties of addictive drugs (Aguiar-Nemer, Toffolo, da Silva, Laranjeira, & Silva-Fonseca, 2013; Lee et al., 2006; Leggio et al., 2011). PYY is an anorexogenic peptide released primarily by L-cells in gut leading to reduced energy intake and body weight (Stadlbauer, Woods, Langhans, & Meyer, 2015). In contrast, ghrelin is an orexigenic hormone released primarily by the stomach that is involved in meal initiation and termination (Wren & Bloom, 2007). Similar to the acute effects of many drugs of abuse, ghrelin and PYY activate neurons directly or indirectly within the mesolimbic dopaminergic pathways and other brain regions linked to the rewarding effects of both food and drugs (Jerlhag et al., 2009; Jerlhag & Engel, 2011; Nakazato et al., 2001; Volkow, Wang, Fowler, Tomasi, & Baler, 2012).

Specific to smoking, among tobacco smokers, weight control is an often cited motivation for smoking in response to the appetite dysregulation that occurs during withdrawal (Borrelli, Spring, Niaura, Hitsman, & Papandonatos, 2001). Changes to appetite hormones during abstinence that regulate hunger and craving may mediate that. Research indicates that ghrelin levels decline in response to acute cigarette smoking for naïve smokers but not habitual smokers (Kokkinos et al., 2007), though with controlled pharmacological delivery of nicotine (2 mg) ghrelin does not change (Kroemer, Wuttig, Bidlingmaier, Zimmermann, & Smolka, 2014; Pilhatsch et al., 2014). Ghrelin is higher in smokers (actively smoking) than nonsmokers (Koopmann et al., 2015). Successful abstinence from nicotine has also been associated with ghrelin declines (Lee et al., 2006) and lower ghrelin during the first 24–48 h of abstinence is associated with longer time to relapse (al'Absi, Lemieux, & Nakajima, 2014). This ghrelin decline from ad libitum smoking to the early relapse period, however, has not consistently been shown (Mutschler et al., 2012). Greater ghrelin decline may be associated with higher nicotine concentrations, though this has been difficult to verify (Pilhatsch et al., 2014). In contrast, PYY has been linked to craving and positive affect during smoking abstinence but not to relapse (al'Absi et al., 2014). While there is conflicting evidence with regards to whether ghrelin and PYY measured during early withdrawal predicts abstinence (Lee et al., 2006; Mutschler et al., 2012), the change from ad libitum to early withdrawal on PYY has not been examined. Similarly, whether the

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ghrelin change reported elsewhere differs from nonsmokers, and is therefore dysregulated, or whether there are important gender differences in either hormone has also not been reported. In a previous report the PYY and ghrelin from the first 24–48 h of confirmed abstinence were used to predict relapse at 28 days. In this report we examine the change from baseline, ad libitum and 48 h abstinent levels of both ghrelin and PYY in male and female nonsmokers and smokers who abstain or relapse over the first one month of abstinence (28 days).

2. Methods

2.1. Participants

Participants who were nonsmokers or chronic smokers who had smoked 10 or more cigarettes per day were recruited into the study. Exclusion criteria included current psychiatric dysfunction (past 1 year), use of prescription medications (except birth control), or obesity (>30% over Metropolitan Life weight tables). Likewise, smokers with carbon monoxide (CO) < 8 ppm at the abstinent lab (second visit) were not included. This resulted in a final sample of 37 nonsmokers (18 female), 37 abstainers (18 female), 54 relapsers (26 female). Diurnal rhythm and menstrual cycle phase were controlled by testing in the follicular phase. The use of street drugs was excluded by self-report using the Alcohol and Other Drug questionnaire.

2.2. Measures

Plasma total ghrelin, PYY, withdrawal symptoms using the Minnesota Withdrawal Scale (Hughes & Hatsukami, 1986), craving, Questionnaire of Smoking Urges brief version (QSU-B: Cox, Tiffany, & Christen, 2001), distress, sleep, and positive affect were collected during the first sample period of ad lib and abstinent laboratory sessions. The Minnesota Withdrawal Scale, distress, craving, and positive affect are all well-validated and commonly used smoking subscales (MNWS, distress, positive affect) or single item rating (craving) from the Subjective State Scale (SSS) (Hughes & Hatsukami, 1986). CO and cotinine was collected during ad lib smoking, 48 h post-cessation, and once per week for the first month of attempted cessation. Cotinine is the major metabolite of nicotine and acts as a biomarker of tobacco exposure. During the screening, demographic (age, marital status, ethnicity, education), psychosocial (distress, positive affect), smoking history (age of onset, cigarettes per day, duration of smoking), alcohol use, anthropometric (height, weight), and nicotine dependence (Fagerström Test of Nicotine Dependence (FTND); Heatherton, Kozlowski, Frecker, & Fagerstrom, 1991) measures were also collected.

2.3. ELISAs

Blood samples (EDTA treated) for cotinine, ghrelin and PYY were collected at the beginning of the session after a rest period. Per instructions by the human ELISA kit manufacturer (EMD Millipore, St. Charles, Missouri; human ghrelin kit # EZGRT–89K; human PYY kit # EZHPYYT66K), the EDTA treated blood samples were treated with AEBSF to insure storage stability (final concentration 1 mg/mL). Following the centrifugation at 2000 x *g* (15 min at $4 \pm 2\,^{\circ}C$), aliquots of plasma were also acidified with HCL (final concentration 0.05N). Both the PYY and ghrelin ELISA kits are sandwich ELISAs using anti-human IgG followed by a second biotinylated antibody for the immobilized IgG-to-molecule complex. Plates were washed, labelled, and quantified via spectrophotometry at 450 nm (corrected at 590 nm) in compliance with kit instructions.

2.4. Study procedures

Each participant attended two laboratory sessions, once during an ad libitum smoking period and once following the first 48 h of abstinence from tobacco. Nonsmoking subjects followed the same pattern of sessions. Participants were instructed to avoid alcohol for 24 h. Food consumption prior to evaluation was scheduled (light lunch one hour prior to the afternoon appointment) and verified via self-report. All sessions were conducted within the same 2 h time frame (12:00–2:00) to control for diurnal variations. For 4 subsequent weeks post-cessation, the smokers returned to the lab for analysis of CO, cotinine and self-reported smoking relapse. Data was collected in both Duluth and Minneapolis, Minnesota.

2.5. Statistical analysis

The primary dependent variables were plasma total ghrelin and PYY levels as well as distress, positive affect, withdrawal symptoms, and craving for cigarettes. Appetite hormonal data were square root transformed to normalize. Smoking for 7 days in a row at any time point after the quit attempt was used to define smoking relapse. The variable "days until relapse" was reported as mean and standard deviation. A series of 3 (smoking group: nonsmokers, abstainers, relapsers) x 2 (sex) x 2 (session: ad libitum, abstinent) repeated measures ANOVA controlling for data collection site were conducted to test our hypotheses. Preliminary correlational analyses found that ghrelin, but not PYY, levels were significantly correlated with age and body mass index (BMI; $ps < 0.05$) and these were used as additional covariates in the ghrelin analysis. Partial eta squared ($_p\eta^2$) is shown for all significant results. Two-way ANOVAs and chi-square tests were conducted to test smoking group and sex differences in demographic and smoking history variables. Correlational analysis was conducted to examine linkages between appetite hormones and subjective measures of craving (F1, F2, and craving item) and smoking history (age of onset, cigarettes per day, FTND). To protect against Type I error, Bonferroni correction was used to readjust the p-value for multiple correlations ($.05/6 = 0.008$). For direct comparison of the significance between two correlations, the Fisher r-to-z transformation was calculated for a one tail test. SPSS v20 was used for statistical analysis. Reported degrees of freedom varied slightly due to occasional missing cases and in analyses with an exclusive focus on the smokers for outcomes such as withdrawal, smoking urges, nicotine dependence (FTND), and smoking history variables.

3. Results

3.1. Participant characteristics

The smoking groups did not differ in marital status, ethnicity, alcohol use, and average nightly sleep over the past week. Consistent with the exclusion criteria, the rate of alcohol use was low in this sample and did not differ across the three groups. For example, 35.1% nonsmokers, 24.3% of abstainers, and 38.3% of relapsers reported at baseline that they never consumer alcohol ($p > 0.10$). There was a significant smoking group x sex interaction in age ($F(2, 122) = 3.17$, $p < 0.05$, $_p\eta^2 = 0.05$) indicating that women were older than men in nonsmokers; however this was not found in smoking groups. Men had higher BMI than women ($F(1, 119) = 5.22$, $p = 0.02$, $_p\eta^2 = 0.04$). A significant main effect of group in years of education ($F(2, 119) = 3.97$, $p = 0.02$, $_p\eta^2 = 0.06$) found that abstainers (those who had not returned to smoking) had lower levels than nonsmokers ($p = 0.02$). Mean CO levels during the abstinent session in smokers were 3.9 ppm (SD = 2.0). Cotinine was reduced by 70% from pre- (mean = 128.2; SD = 98.4) to post-quit (mean = 39.4; SD = 38.1).

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