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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 ($p < 0.01$). While nonsmokers and abstainers showed no change in ghrelin across the initial 48h, relapsers declined ($p < 0.01$). With PYY, relapsers increased ($p < 0.05$) across the early abstinence phase. PYY and ghrelin may be useful predictors of relapse, specifically in reference to early withdrawal.

Keywords

Peptide YY; Ghrelin; Craving; Withdrawal; Nicotine dependence; Relapse

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; Hillemeier 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 anorexigenic 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

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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 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 AEBSEF to insure storage stability (final concentration 1 mg/mL). Following the centrifugation at 2000 $\times g$ (15 min at $4 \pm 2^\circ \text{C}$), 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) $\times$ 2 (sex) $\times$ 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; $p < 0.05$) and these were used as additional

covariates in the ghrelin analysis. Partial eta squared (η^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 ($F(2, 122) = 3.17, p < 0.05, \eta^2 = 0.05$) indicating that women were older than men in nonsmokers; however this was not found in smoking groups. Men had higher BMI than women ($F(1, 119) = 5.22, p = 0.02, \eta^2 = 0.04$). A significant main effect of group in years of education ($F(2, 119) = 3.97, p = 0.02, \eta^2 = 0.06$) found that abstainers (those who had not returned to smoking) had lower levels than non - smokers ($p = 0.02$). 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).

The mean days until relapse was 7.2 (SD = 6.4; median = 4.4 days). Abstainers and relapsers did not differ in the age of onset of smoking, average cigarettes per day, duration of cigarette use, nicotine dependence, and CO. Male relapsers had greater CO levels than female relapsers; this sex difference was not found in abstainers ($F(1, 67) = 6.05, p = 0.02$).

3.2. Subjective craving and withdrawal symptoms

There was a significant main effect of smoking group in distress ($F(2, 128) = 4.09, p = 0.019, \eta^2 = 0.06$) with higher levels in relapsers than in nonsmokers ($p = 0.006$). A significant session main effect in positive affect ($F(1, 128) = 7.36, p < 0.008, \eta^2 = 0.05$) reflected decreased levels from the ad libitum to abstinence session. Smokers who relapsed had higher levels of craving ($F(1, 93) = 8.35, p = 0.005, \eta^2 = 0.08$) and appetitive smoking urges (F1: $F(1, 93) = 6.78, p = 0.01, \eta^2 = 0.07$) than abstainers. There was a decrease in appetitive smoking urges (F1) in the abstinent session ($F(1, 93) = 14.64, p = 0.001, \eta^2 = 0.14$). For the smokers MNWS scores increased from ad libitum to abstinent session ($F(1, 101) = 5.38, p < 0.05, \eta^2 = 0.05$) but no other factors (main effects or interactions)

were significant. Smoking urges (aversive) based on the QSU-B F2 scale did not change by time, relapse status, gender or an interaction.

3.3. Appetite hormones

Ghrelin showed a significant main effect of smoking group ($F(2, 92) = 3.86, p = 0.02, \eta^2 = 0.08$). Bonferroni corrected pairwise comparisons indicated that the relapsers had higher ghrelin than the nonsmokers ($p = 0.02$) but no other comparison was significant. A main effect of sex indicated that women had higher ghrelin than men ($F(1, 92) = 8.38, p = 0.005, \eta^2 = 0.08$). There was no main effect of session, but the main effect of smoking group was further qualified by a smoking group by session interaction ($F(2, 92) = 3.11, p = 0.05, \eta^2 = 0.06$). Follow-up *t*-tests for session within each relapse group (collapsing across gender) indicate that relapsers showed a decrease ($p = 0.03$) in ghrelin across sessions while nonsmokers and abstainers showed no change (Fig. 1). Though non-significant from both nonsmokers and relapsers, the abstainers were midway between the other two groups (Table 1).

The analysis of PYY indicated that there was no main effect of session or sex. There was, however, a main effect of group ($F(2, 106) = 3.04, p = 0.05, \eta^2 = 0.05$) where relapsers had higher PYY than nonsmokers ($p = 0.05$). No other group comparison was significant. Furthermore, there was a significant smoking group by session interaction ($F(2, 101) = 4.86, p = 0.01, \eta^2 = 0.06$; see Fig. 2). The post hoc *t*-tests for session within each relapse group (collapsing across gender) indicated that the relapsers showed an increase in PYY across sessions ($p = 0.001$) whereas nonsmokers and abstainers showed no change. No other interactions were significant.

3.4. Correlational analysis

Exploratory correlational analyses were conducted in the entire smoking group, in each smoking status (abstainers, relapsers) and in each sex (female and male smokers) to explore linkages between appetite hormones and self-report measures. In the smokers regardless of relapse status, greater ghrelin in the abstinent session was associated with earlier age of onset of smoking ($r(80) = -0.23, p = 0.04$). Ghrelin levels demonstrated a positive correlation with average cigarettes per day in relapsers ($r(42) = 0.32, p = 0.04$) but not in abstainers ($r(29) = 0.15, p = 0.44$). No other significant correlations with ghrelin were found. PYY in the abstinent session was inversely linked with FTND scores in the relapsers ($r(48) = -0.35, p = 0.01$) but not the abstainers ($r(32) = 0.06, p = 0.73$), and in female smokers ($r(41) = -0.44, p = 0.004$) but not in male smokers ($r(46) = -0.05, p = 0.72$). PYY in the abstinent session was inversely linked with QSU factor 2 in the relapsers ($r(48) = -0.36, p = 0.01$) but not in abstainers ($r(32) = -0.05, p = 0.77$). This correlation was also found in the female smokers ($r(41) = -0.35, p = 0.03$) but not in male smokers ($r(46) = -0.12, p = 0.43$). Similarly, PYY in the abstinent session was negatively correlated with QSU factor 1 in relapsers ($r(48) = -0.33, p = 0.02$) but not in abstainers ($r(32) = 0.29, p = 0.10$). There was a negative correlation between PYY and craving in the abstinent session among relapsers ($r(48) = -0.38, p = 0.007$) but this relationship was in the opposite direction among abstainers ($r(32) = 0.35, p = 0.05$). With the more stringent *p*-value correction ($p = 0.008$), only the correlations between PYY and female's FTND and relapsers' craving remain significant.

The comparison between correlations for the PYY and craving for relapser and abstainers was significant ($p = 0.0006$), as was the comparisons between PYY and FTND scores for males versus females ($p = 0.03$) (Table 2).

4. Discussion

This study demonstrated that there was a significant decrease in ghrelin during the initial withdrawal period while PYY increased, both preferentially for the relapsers. In this analysis, the comparison of all three groups (nonsmokers, abstainers and relapsers) was critical in clarifying patterns that were not evident in studies that examine only nonsmokers and abstainers (Mutschler et al., 2012) and demonstrating that dysregulation in these hormones is most significant in those who relapse. In this way, our results do not differ from those showing no effect of abstinence on ghrelin (Mutschler et al., 2012), though it highlights that abnormal *changes* in ghrelin accompanies relapse. While the work by Mutschler et al. (2012) showed that 24 h abstinence in smokers confirmed to remain abstinent ($n = 11$) do not differ from nonsmokers ($n = 10$), as we show here, but Mutschler did not include a sample of individuals who subsequently relapsed. Our results suggest that stratifying by relapse status is critical. Likewise, higher ghrelin in relapsed smokers is similar to what is observed with alcoholics at the 72 h abstinent, but not later, phase of withdrawal (Leggio et al., 2012). Given the quasi-experimental nature of this observational study, it is not possible to determine causality but the time course suggests that the change in hormones preceded the determination of relapse for both PYY and ghrelin. Causal inference is not without precedence as others have demonstrated a clear causal link with ghrelin in relation to alcohol use via direct manipulation of peripheral ghrelin levels (Leggio et al., 2014).

As expected, those who relapsed had more distress, craving and smoking urges. Consistent with previous publications (al'Absi et al., 2014), PYY was consistently, and negatively, correlated with smoking patterns, craving, and urges, but then only for the relapsing smokers and smoking women. Nearly the same relationship was seen between PYY and craving for abstainers, but in this case in the positive direction. The differences between the 2014 study (PYY associated negatively with smoking urges; al'Absi et al., 2014) and the current study (marginally positive correlation with craving in abstainers but negative in relapsers) may be due to differences in sample size, the use of baseline versus forced abstinence samples, or differences in statistical model building. Further, the use of baseline samples in the 2014 study was from a combined sample of two studies, one of which did not have an ad libitum baseline, which precluded the splitting into relapse or abstain for the regression analysis. Thus this study examines a comparison of baseline ad libitum smoking to an abstinent period, information that was not assessed in the 2014 study. However, in addition to supporting past studies specific to smoking (al'Absi et al., 2014), these results also further support the hypothesis that PYY maybe protective and have antidepressant qualities (Painsipp, Herzog, Sperm, & Holzer, 2011).

Finally, it has been suggested that subjective measures of taste (sweet preference in humans) and subtyping use patterns based on psychological components of urges or craving can provide important clues to the interrelationships between appetite mediators in alcoholism

(Hillemacher et al., 2007; Leggio et al., 2011), a concept that may well prove useful in nicotine addiction in future studies. As stress impacts sweet taste perception in controlled laboratory testing, it will be critical to determine whether the psychological stress of withdrawal or changes in appetite mediators account for the proposed relationship.

One limitation of this study is the use of one hormone sample per session and the use of total ghrelin. Given that other appetite hormones in smokers are sensitive to experimental manipulation of stress (al'Absi, Lemieux, Nakajima, Hatsukami, & Allen, 2015), it will be important to assess ghrelin and PYY across similar manipulations. Likewise, although total ghrelin correlates with the biologically active acetylated ghrelin (Tong et al., 2013) the current results are based on total ghrelin and, thus, we cannot disentangle active from inactive forms. However, the belief that des-acyl ghrelin is non-functional compared to acylated ghrelin has been challenged and more investigators are therefore using total ghrelin in their studies. For example, total ghrelin, but not acylated ghrelin, has been used by investigators studying the effects of human olfaction on brain, gastric, and/or metabolic functions (Saliakellis et al., 2015). In psychological studies where both have been measured, it is total, but not acylated ghrelin, that correlates with hedonic responses (Trellakis et al., 2011). Total ghrelin has been found to be important in anorexia (Germain et al., 2010; Ogiso, Asakawa, Amitani, & Inui, 2011) though not stress (Kiessl & Laessle, 2016). Finally, within studies of addiction total ghrelin has also been used either in isolation (al'Absi et al., 2014; Bouros et al., 2006; Kokkinos et al., 2007) or in contrasting total and acylated ghrelin (Koopmann et al., 2015). The effects of nicotine, and its absence in addicted smokers, on both forms of ghrelin (acylated ghrelin and des-acyl ghrelin) are complex, dynamic, and likely affecting both peripheral (des-acyl) and central (acylated) functions. The indirect brain effects of peripheral des-acyl ghrelin via peripheral mediators are, as yet, unclear but evidence has been presented that des-acyl ghrelin affects the arcuate nucleus, a brain region clearly linked to addiction, via non-ghrelin receptor pathways (Fernandez et al., 2016). Given this, it would be premature to focus an entire literature (smoking or addiction and ghrelin) solely on acylated ghrelin but measuring only total ghrelin limits our ability to assess correlations between ghrelin forms (acyl and des-acyl) or with other variables (such as craving). In this regard the choice to measure total ghrelin is a major limitation of this study. Future studies of ghrelin and smoking would therefore benefit from the measurement of both des-acyl and acylated ghrelin, but not exclusively acylated ghrelin, to allow for more rich and complex modeling of these factors. As PYY and ghrelin are sensitive to food intake, correction with blood glucose levels at the time of testing would also be preferable, but was not conducted here. Finally, here a conservative definition of relapse was used, no urinalysis was used to document drug use, and no direct assessment of alcohol intake prior to each session. These too are important limitations, for example given known associations between smoking and alcohol use during cessation attempts (Cook et al., 2012) as well as acyl-ghrelin and alcohol craving (Koopmann et al., 2012), and it will be important to examine other more liberal definitions of relapse and incorporate urinalysis and breathalyzer assessment into the study protocol.

Despite these limitations, the current report demonstrates that ghrelin and PYY do change across the stress of early tobacco cessation, but only when relapse status is included in the analysis and compared to nonsmokers. We further supported previous demonstration that

PYY negatively correlates with smoking urges and craving, but we add the additional information here that these effects are absent during ad libitum smoking. Evidence is beginning to mount that ghrelin is related to smoking history (age of onset) in this study and relapse in previous publications (al'Absi et al., 2014), though there are inconsistencies between studies on the issue of relapse. Whether these cross study differences and the lack of significance between abstainers and either nonsmokers or relapsers are due to sample size differences remain to be seen. Further examination of how these hormones relate to central dopamine and opiate reward pathways in future studies will be important.

In conclusion, we set out to examine how PYY and ghrelin change across the initial smoking abstinence period by contrasting the changes seen in smokers who remain abstinent, smokers who ultimately relapse, and nonsmokers studied across the same time frame. We demonstrated that for relapsers, but not abstainers or nonsmokers, PYY increases and ghrelin decreases. We further show that PYY correlates with nicotine dependence (FTND), aversive smoking urges (QSU–B F2), and nicotine craving for relapsers but not abstainers and that PYY correlated with nicotine dependence for women but not men. These results demonstrate the importance of expanding the contrast of smoking status (smoker, nonsmoker) to include relapse status (abstaining versus relapsing) and sex. The results also highlight the need for future studies that can carefully measure and contrast acylated and des-acyl ghrelin to PYY and explain further the clinical meaning of these factors.

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Fig. 1. Ghrelin is depicted (raw values) for ad libitum smoking and 48 h post-cessation across the three groups. The analysis indicates that relapsers, but no other group, show a decline ($p = 0.03$).

Fig. 2.

PYY is depicted (raw values) for ad libitum smoking and 48 h post-cessation across the three groups. The analysis indicates that relapsers increased but non-smokers and abstainers had no change in PYY ($p = 0.001$).

Table 1
Hormone levels across ad libitum (normal pattern smoking) and abstinence sessions.

		Nonsmokers Mean (SE)	Abstainers Mean (SE)	Relapsers Mean (SE)	Female Mean (SE)	Male Mean (SE)
Ghrelin ^a	Ad Libitum	403.41 (42.23)	480.55 (47.68)	586.83 (36.20)	552.70 (35.15)	427.83 (34.25)
	Abstain	444.41 (37.13)	448.32(41.92)	526.43(31.83)	538.33 (30.90)	407.78(30.12)
PYY	Ad Libitum	73.52(6.79)	74.25 (7.58)	78.48 (6.05)	75.68 (5.70)	75.14(5.45)
	Abstain	69.77(5.67)	79.91 (6.33)	88.94(5.05)	80.06 (4.76)	79.02 (4.56)

^a Ghrelin and PYY are shown in raw values (pg/ml) here for descriptive purposes. All analyses were conducted on normalized values.

Table 2

Correlations between appetite mediators, smoking history and craving.

	Abstainers		Relapsers		Females		Males	
	r	p-value	r	p-value	r	p-value	r	p-value
PYY								
FTND	0.06	0.729	<i><u>-0.35</u></i>	<i><u>0.014</u></i>	-0.44	0.004	-0.06	0.715
Cigarettes per day	0.34	0.061	-0.15	0.328	0.00	0.997	0.10	0.521
Age of onset	0.11	0.554	-0.12	0.406	0.02	0.890	-0.07	0.627
QSU-B F1	0.29	0.104	<i><u>-0.33</u></i>	<i><u>0.021</u></i>	-0.25	0.109	0.06	0.674
QSU-B F2	-0.05	0.771	<i><u>-0.36</u></i>	<i><u>0.012</u></i>	<i><u>-0.35</u></i>	<i><u>0.025</u></i>	-0.12	0.431
Craving	<i><u>0.37</u></i>	<i><u>0.035</u></i>	-0.41	0.004	-0.15	0.340	-0.05	0.746
Ghrelin								
FTND	-0.16	0.392	-0.01	0.922	-0.13	0.447	-0.28	0.086
Cigarettes per day	0.15	0.440	<i><u>0.32</u></i>	<i><u>0.041</u></i>	0.19	0.273	0.12	0.468
Age of onset	-0.24	0.248	-0.26	0.070	-0.26	0.123	-0.20	0.221
QSU-B F1	-0.09	0.677	0.17	0.232	0.20	0.240	0.18	0.267
QSU-B F2	-0.16	0.446	0.19	0.193	0.25	0.132	0.06	0.715
Craving	-0.06	0.786	0.24	0.094	0.224	0.182	0.23	0.170

The values in underline italics are significant at $p = 0.05$ while the bolded items are significant after Bonferroni correction to $p < 0.008$.