The Relationship of Serum Leptin and Ghrelin Levels with Craving and Withdrawal in Opioid Use Disorder

ABSTRACT

Objective: This study aims to investigate plasma levels of leptin, acyl ghrelin, and unacylated ghrelin during heroin withdrawal in patients with opioid use disorder (OUD) with regard to the relationship of these levels with craving and their changes over time.

Methods: This study included 28 male patients diagnosed with OUD according to DSM-5 diagnostic criteria who received inpatient rehabilitation. The control group included 28 healthy male volunteers with characteristics similar to the patient group. Plasma leptin, acyl ghrelin, and unacylated ghrelin levels of the patients were measured 3 times throughout the study by collecting blood on the first day, the seventh day at the end of the detox, and the twenty-first day. Blood was collected only once from the control group to determine their plasma leptin, acyl ghrelin, and unacylated ghrelin levels.

Results: Our study did not determine any statistically significant differences between patients with OUD and healthy controls with regard to plasma leptin, acyl ghrelin, and unacylated ghrelin levels on the first, seventh, and twenty-first days of withdrawal. Plasma levels of leptin, acyl ghrelin, and unacylated ghrelin did not significantly correlate with craving scores.

Conclusion: This study does not support the hypothesis that plasma leptin, acyl ghrelin, and unacylated ghrelin levels are markers in those with OUD. Further research, particularly in humans, is recommended to replicate and expand on the findings of the current literature.

Keywords: Opioid-related disorders, craving, ghrelin, leptin, withdrawal

Introduction

Opioid use disorder (OUD) is the inability to stop using larger amounts of the opioid over a longer period of time without medical reasons.¹ It is defined as a chronic, relapsing disorder characterized by neurobiological changes and represents a strong desire or urge to use opioids.² The most important problem encountered in the treatment of OUD is the higher rates of relapse.³ Therefore, the etiology and risk factors of addiction and underlying reasons behind the urge to substances should be investigated.⁴ Studies in patients with OUD suggest that neuroendocrine changes during opioid withdrawal may contribute to clinical symptoms and relapse.⁵,⁶ It has been revealed that leptin, which is a hormone secreted mainly by white adipose tissue, reduces nutrient intake and increases energy expenditure, may have effects on substance craving in rats.⁷ In a study, it was reported that leptin levels and body mass index (BMI) increased after 1 year of treatment in opioid addicts.⁸ Ghrelin is a polypeptide hormone synthesized by enteroendocrine cells of the gastrointestinal tract, mainly in the stomach. It induces growth hormone secretion, appetite, and lipogenesis.⁹ Ghrelin signaling plays a critical role in mediating the behavioral and biochemical effects of substance abuse, which is essential for the development of dependence, particularly for alcohol, nicotine, and stimulants.¹⁰ Evaluating levels of these hormones in individuals with OUD can provide a source for relapse and recurrence studies and play a key role in studies on substance abuse treatment. This...
study aimed to determine serum leptin and ghrelin levels during the withdrawal period and 21-day follow-up period in patients with OUD and show if there was a change during the withdrawal period. After all, it was aimed to reveal whether leptin and ghrelin are biological markers related to craving and withdrawal in OUD patients. Our H0 hypothesis is that plasma leptin, acylated ghrelin, and unacylated ghrelin levels do not correlate with craving during opiate withdrawal and do not change over time. Our H1 hypothesis is that plasma leptin, acylated ghrelin, and unacylated ghrelin levels increase over time during opiate withdrawal and correlate with craving.

Methods

Participants and Biochemical Analysis

This study was conducted between January 2019 and July 2019 at the Akdeniz University Alcohol and Substance Abuse Research and Application Center. Power analysis was not performed for the sample size. It is planned to take 50 patients and 50 controls. In total, 51 male patients diagnosed with OUD according to the DSM-5 diagnostic criteria were included in the study. However, 23 of these patients voluntarily left our clinic without completing the study. Finally, a total of 28 patients were included in the study. The control group consisted of 28 healthy male subjects who had similar characteristics to the patient group and had no history of chronic disease (severe inflammatory, cardiac, endocrine, renal or hepatic disease, etc.). Having any chronic disease (severe inflammatory, cardiac, endocrine, renal or hepatic disease, etc.). Having any major psychiatric disorder accompanying OUD according to the Structured Clinical Interview for DSM-5 (SCID-5) (schizophrenia, bipolar affective disorder, major depression, etc.).

The inclusion criteria for the study were as follows:

- Being between 18 and 65 years of age.
- Meeting the DSM-5 criteria for OUD.

The exclusion criteria for the study were as follows:

- Having any psychiatric disorder (bipolar affective disorder, major depression, etc.).
- Taking a drug that affects metabolism (other than buprenorphine-naloxone).
- Smoking up to 2 hours before taking blood.
- Non-opiate substance and alcohol use.

The Sociodemographic Data Form and Structured Clinical Interview for DSM-5 were administered to both patient and control groups.11 In addition, the Substance Craving Scale (SCS) was administered to the patient group. SCS is an adaptation of the Penn Alcohol Craving Scale, which evaluates the desire to use alcohol, for addicts using non-alcohol substances. The validity and reliability study of the scale was conducted by Evren et al.12 It consists of 5 items, and each item is scored between 0 and 6 points. The total score that can be obtained from the scale is between 0 and 30. The Cronbach’s alpha value for the scale was 0.84.12 It has been reported that 48-72 hours of fasting lead to an increase in circulating ghrelin levels, and short-term fasting results in a rapid and marked decline in leptin levels out of proportion to the loss of fat mass.13,14 Leptin and ghrelin (acyl and unacyl forms) levels were measured 3 times during the study—on the first day, seventh day (at the end of detox treatment), and twenty-first day. They were measured once in the control group. Blood samples were taken from 08.00 AM to 08.30 AM after fasting of at least 10-12 hours. The participants were asked not to smoke for at least 2 hours before blood collection. Blood samples were centrifuged for 15 minutes at 4000 rpm and then stored at −80ºC until analysis. Serum leptin levels (Human Leptin LEP ELISA kit, YL BIONT, Shanghai), serum acylated ghrelin levels (Human Acylated ghrelin (AG) ELISA kit, YL BIONT, Shanghai), and serum unacylated ghrelin levels (Human unacylated ELISA kit, YL BIONT, Shanghai) were measured by the ELISA method as in similar studies.15,16 Analysis results were calculated as ng/mL for leptin and acylated ghrelin and ng/L for unacylated ghrelin according to the manufacturer’s instructions.

Statistical Analysis

Statistical analysis was performed using SPSS version 22.0 (IBM Corp; Armonk, NY, USA). The Kolmogorov–Smirnov and Shapiro–Wilks tests were used to check if the quantitative data were normally distributed. While the independent samples t-test was used to compare normally distributed data between groups, the Mann–Whitney U-test was used to compare non-normally distributed data between groups. The Friedman test was used for intragroup comparisons. The Wilcoxon test was used for post hoc analysis of nonparametric dependent variables, and the comparison results were interpreted according to the Bonferroni correction. Spearman correlation coefficient was used for correlation analysis of continuous variables with the non-normal distribution. Descriptive statistics were expressed as mean (standard deviation) in parametric tests. Median, 25% and 75% percentile values (Q1–Q3) were given in nonparametric tests. P-values below .05 were considered statistically significant.

Results

In this study, 28 patients diagnosed with OUD according to the DSM-5 diagnostic criteria and 28 healthy individuals have participated in the study. All patients and healthy controls were male. The mean age of the patient group was 26.04 (SD = 3.99) years. Of the patient group, 85.7% (n = 24) were single, 10.7% (n = 3) were married, and 3.6% (n = 1) were divorced. The mean age of the control group was 26.07 (SD = 4.64) years. Of the control group, 71.4%...
(n = 20) were single, 25.0% (n = 7) were married, and 3.6% (n = 1) were divorced. Age and BMI were compared between the patient and control groups using the independent samples t-test. There were no statistically significant differences between the patient and control groups in terms of age and BMI (Table 1). The mean BMI value of the patient group was 22.36 (SD = 2.78) kg/m² on the first day, 23.03 (SD = 2.99) kg/m² on the seventh day, and 23.17 (SD = 2.88) kg/m² on the twenty-first day, respectively. The mean BMI of the control group was 22.15 (SD = 2.47) kg/m². In the patient group, there were statistically significant differences between the mean BMI values on the first day and seventh day and between the mean BMI values on the first day and twenty-first day. In the patient group, the mean BMI value was significantly lower on the first day than on the seventh day and twenty-first day (chi-square = 14.00; df = 2; P < .05). In addition, there was a negative correlation between the mean BMI value on the 21st day and the mean plasma unacylated ghrelin level on the 21st day in the patient group (r = −0.400; P = .035) (Table 1).

In the patient group, the mean age of onset of opioid was 19.43 (SD = 3.02) years, and the mean dose of opioid use was 1.07 (SD = 0.56) g/day. The method of opioid use was the inhalation through heated aluminum foil in 75% (n = 21) of the patients, whereas 17.9% (n = 5) used intravenously and 7.1% (n = 2) used both methadone and buprenorphine, LSD, etc.). The control group had no history of substance use.

The mean duration of opioid use was 6.61 (SD = 4.52) years. Of the patient group, 20 had a history of cannabis use, 12 had a history of cocaine use, and 14 had a history of other substance use (ecstasy, bensid, LSD, etc.). The control group had no history of substance use.

In the patient group, the median plasma leptin level was 5.62 ng/mL on the first day, 6.31 ng/mL on the seventh day, and 5.78 ng/mL on the twenty-first day, respectively. The median plasma acylated ghrelin level was 1.78 ng/mL on the first day, 1.84 ng/mL on the seventh day, and 1.99 ng/mL on the twenty-first day, respectively. The median plasma unacylated ghrelin level was 269.42 ng/L on the first day, 234.42 ng/L on the seventh day, and 301.26 ng/L on the twenty-first day, respectively. In the control group, the median plasma leptin level was 6.13 ng/mL, the median plasma acylated ghrelin level was 1.89 ng/mL, and the median plasma unacylated ghrelin level was 276.81 ng/L. The median plasma leptin, acylated ghrelin, and unacylated ghrelin levels were compared between the patient and control groups using the Mann–Whitney U-test. There were no statistically significant differences in the median plasma leptin, acylated ghrelin, and unacylated ghrelin levels between the patient and control groups (Table 2).

In the patient group, the median plasma leptin, acylated ghrelin, and unacylated ghrelin levels on the first day, seventh day, and twenty-first day were compared to each other using the Friedman test. There were no statistically significant differences between them (Table 3).

The median SCS score was 17.00 on the first day, 6.50 on the seventh day, and 4.50 on the twenty-first day. There were statistically significant differences between them (Friedman chi-square = 42.769; df = 2; P < .001). Accordingly, it gradually decreased during the withdrawal period. There were no statistically significant relationships between

### Table 1. Comparison of Clinical Characteristics of Patients and Control Groups

|                  | Patient (n = 28), Mean (SD) | Control (n = 28), Mean (SD) | P* |
|------------------|-----------------------------|-----------------------------|----|
| Age (year)       | 26.04 (3.95)                | 26.07 (4.64)                | .975 |
| BMI (kg/m²)      | 22.36 (2.78)                | 22.15 (2.47)                | .765 |

Abbreviation: BMI, body mass index.

*Independent samples t-test.

### Table 2. Comparison of Plasma Leptin, A cylated Ghrelin, and Unacylated Ghrelin Levels Between Patient and Control Groups

|                    | Patient median (Q1-Q3) | Control median (Q1-Q3) | P* |
|--------------------|------------------------|------------------------|----|
| Leptin 1 (ng/mL)b  | 5.62 (4.47-10.09)      | 6.13 (4.94-11.18)      | .287 |
| Leptin 2 (ng/mL)b  | 6.31 (4.74-10.40)      | 6.13 (4.94-11.18)      | .544 |
| Leptin 3 (ng/mL)b  | 5.78 (4.64-9.62)       | 6.13 (4.94-11.18)      | .342 |
| Acylated ghrelin 1 (ng/mL)b | 1.78 (1.36-3.04) | 1.89 (1.66-3.44) | .225 |
| Acylated ghrelin 2 (ng/mL)b | 1.84 (1.27-2.89) | 1.89 (1.66-3.44) | .245 |
| Acylated ghrelin 3 (ng/mL)b | 1.99 (1.20-2.75) | 1.89 (1.66-3.44) | .179 |
| Unacylated ghrelin 1 (ng/L)b | 269.42 (202.17-493.92) | 276.81 (223.05-488.97) | .694 |
| Unacylated ghrelin 2 (ng/L)b | 234.42 (197.59-484.04) | 276.81 (223.05-488.97) | .310 |
| Unacylated ghrelin 3 (ng/L)b | 301.26 (229.02-412.92) | 276.81 (223.05-488.97) | .909 |

*Mann–Whitney U-test.

*Leptin 1: 1st day, leptin 2: 7th day, leptin 3: 21st day; acylated ghrelin 1: 1st day, acylated ghrelin 2: 7th day, acylated ghrelin 3: 21st day; unacylated ghrelin 1: 1st day, unacylated ghrelin 2: 7th day, unacylated ghrelin 3: 21st day.

### Table 3. Plasma Leptin, A cylated Ghrelin, and Unacylated Ghrelin Levels of Patients

| Withdrawal period | First day | Seventh day | 21st day | P* |
|-------------------|----------|-------------|---------|----|
| Leptin (ng/mL), Median (Q1-Q3) | 5.62 (4.47-10.09) | 6.31 (4.74-10.40) | 5.78 (4.64-9.62) | .965 |
| Acylated ghrelin (ng/mL), Median (Q1-Q3) | 1.78 (1.36-3.04) | 1.84 (1.27-2.89) | 1.99 (1.20-2.75) | .898 |
| Unacylated ghrelin (ng/L), Median (Q1-Q3) | 269.42 (202.17-493.92) | 234.42 (197.59-484.04) | 301.26 (229.02-412.92) | .991 |

*Friedman test.

Substance Craving Scale first day — Substance Craving Scale seventh day (P < .001) (Bonferroni correction); Substance Craving Scale first day — Substance Craving Scale twenty-first day (P < .001) (Bonferroni correction); and Substance Craving Scale seventh day – Substance Craving Scale twenty-first day (P < .184) (Bonferroni correction).
the median plasma leptin, acylated ghrelin and unacylated ghrelin levels and the median SCS scores (Table 4).

**Table 4. Relationship Between Craving and Leptin, Acylated Ghrelin, and Unacylated Ghrelin Levels**

|                      | SCS1* | SCS2* | SCS3* | P     |
|----------------------|-------|-------|-------|-------|
| Leptin 1*            | 0.000b |       |       | .999  |
| Leptin 2*            | 0.158b |       |       | .422  |
| Leptin 3*            | 0.240b |       |       | .219  |
| Acylated ghrelin 1*  | 0.067b |       |       | .735  |
| Acylated ghrelin 2*  | 0.246b |       |       | .206  |
| Acylated ghrelin 3*  | 0.148b |       |       | .451  |
| Unacylated ghrelin 1*| 0.015b |       |       | .939  |
| Unacylated ghrelin 2*| 0.154b |       |       | .433  |
| Unacylated ghrelin 3*| 0.044b |       |       | .825  |

*Values are the correlation coefficient. All P-values > .05.

**Discussion**

In our study, there was no statistically significant difference in plasma leptin, acylated ghrelin, and unacylated ghrelin levels between patients and healthy controls. Our study also showed that there were no statistically significant correlations between craving and plasma leptin, acylated ghrelin, and unacylated ghrelin levels.

The levels of leptin and ghrelin in addiction have been studied on different substances. In a study conducted on patients with alcohol dependence, no statistically significant difference was found in leptin, ghrelin and prolactin levels between the patient group and the control group. The leptin and prolactin levels of the patients with alcohol dependence did not change significantly within days. There was a statistically significant decrease in the ghrelin levels of patients with alcohol dependence between the 0th day and the 28th day and between the 1st and the 28th day. In another study, leptin levels were found to be significantly higher in alcohol-dependent patients than in the control group. Also, levels were positively correlated with craving scores.

In our study, another study involving 14 heroin-dependent individuals and 17 healthy controls found that there was no statistically significant difference in serum leptin levels between the heroin and control groups. It was also determined that serum leptin levels increased significantly after 1 year of methadone maintenance treatment in the heroin group compared to the control group. These 2 studies investigating leptin levels in heroin addicts revealed that leptin levels increased after methadone maintenance treatment. However, they did not examine whether there was a relationship between leptin levels and craving. These studies found that leptin levels were lower in the opioid-dependent group than in the control group. These increases seen after methadone treatment suggest that opioid agonist treatment may have an effect on these hormones. It is also suggested that there may be a relationship between leptin receptors and buprenorphine.

A possible factor that can lead to hypoleptinemia in addicts has decreased cortisol levels. Since glucocorticoids are strong stimulants of leptin expression in human adipose tissue; leptin levels may decrease due to their deficiencies. Moreover, the fact that there was no change in leptin levels, although there was a change in weight during the withdrawal period in our study, suggests that leptin production may be affected by heroin or its metabolites. In another study conducted with 12 heroin addicts and 8 healthy controls, neuroendocrine changes and self-reported cravings for heroin in heroin addicts were evaluated on different days of the first month (the 3rd, 10th, and 30th day), similar to our study. It was found that leptin levels were significantly lower during the early withdrawal period (the 3rd and 10th day) in the heroin group than in the control group, but leptin levels returned to normal on the 30th day. It was
also determined that there was no statistically significant relationship between plasma leptin levels and craving.\textsuperscript{5}

The fact that ghrelin interacts with both reward processing and stress pathways suggests that it plays a role in addictive behaviors.\textsuperscript{36} Ghrelin administration has been found to increase heroin-seeking behavior in rats. In contrast, ghrelin receptor antagonist administration has been shown to have no significant effect on existing heroin-seeking behavior or on hunger-induced heroin-seeking behavior.\textsuperscript{37} Previous investigations support a connection between the ghrelin system and alcohol, stimulants, and tobacco use in both animals and humans, while the research on opioids and cannabis is rare. A study investigating plasma acylated and unacylated ghrelin levels in patients with OUD has not been available in the literature.\textsuperscript{38} Although rat studies have shown that ghrelin may have a role in OUD, our study found that there were no statistically significant differences in plasma acylated and unacylated ghrelin levels between patients with OUD and healthy controls. In addition, our study also showed that there were no statistically significant relationships between opioid craving and plasma acylated and unacylated ghrelin levels.

Our study has some limitations. Firstly, all the participants in the sample group were male. Secondly, the fact that the SCS is based on self-report might prevent the objective evaluation of craving. Finally, the patients were receiving buprenorphine-naloxone treatment. It can suppress withdrawal symptoms and affect leptin and ghrelin levels. This is an important limitation of our study. Our study has some strengths. First, our study contained a control group. Second, our study is the first study to investigate plasma acylated and unacylated ghrelin levels in opioid-dependent patients. Finally, our study is the most extensive study on this subject in the literature.

This study has not supported the hypothesis that plasma leptin, acylated ghrelin, and unacylated ghrelin levels are a determinant in patients with OUD. On the other hand, there is a need for studies evaluating the relationship between these hormones and treatments for reducing craving and withdrawal symptoms.

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