Plasma Ghrelin Concentration and Obesity: The Effect of Smoked Drugs

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Abstract

Cannabis remains one of the most widely abused drugs worldwide. This study was carried on 107 non-diabetic males, 63 were smoking cannabis in order to examine the effect of smoking cannabis on the level of ghrelin concentration in plasma and to explain the cause of obesity in cannabis smoking. The studied group (63 subjects) were selected according to their BMI (body mass index) and subgrouped to: Group 1: Included 34 obese persons with BMI >30kg/m². Group 2: Included 29 non-obese persons with BMI <30kg/m² and they were instructed not to use any form of cannabis for 4 weeks and to come again for recheck for cannabis level in urine. Beside 44 healthy persons (negative for cannabis in urine) were selected as control groups and were divided into two groups according to their BMI: Group 3: Included 23 obese persons with BMI >30kg/m². Group 4: Included 21 non-obese persons with BMI <30kg/m². Complete medical examinations and the BMI was calculated, plasma glucose level was estimated, human plasma ghrelin was measured in a fasting state, and urinary cannabis level was estimated by the Emit II plus Cannabinoid Assay.

The results showed that, Fasting plasma ghrelin concentrations were low in obese subjects (groups 1 & 3) compared with non obese subjects (groups 2 & 4). While plasma ghrelin concentrations were higher in all patients using cannabis (groups 1 & 2) compared with control subjects (groups 3 & 4). Plasma ghrelin level was negatively correlated with BMI in groups 1 & 2 with correlation coefficient \( r=-0.8549, -0.81177 \) respectively. Also, the results showed that, plasma ghrelin was positively correlated with the A9-THC urine level in the first visit in both groups 1 & 2 with correlation coefficient \( r=0.9150 \) & 0.8889 respectively.

These findings concluded that ghrelin is a potentially important peripheral signal to the brain stimulating food intake in cannabis smoking. Also the results provide an evidence for a functional relationship between urinary cannabinoid levels and plasma ghrelin concentration in the control of food intake among cannabis smoking and their relation to the obesity.

Key Words: Cannabis – Ghrelin – Obesity.

Introduction

The appetite-stimulating effects of the cannabis plant (Cannabis sativa) appear to be effected through the incentive and rewarding properties of foods. The physiological control of eating behavior is extremely complex, involving a balance of both central and peripheral neurotransmitters and neuropeptides that interact to stimulate or inhibit food intake [1]. The endocannabinoid system, consisting of two cannabinoid receptors (CB1 and CB2) and the endogenous ligand anandamide [arachidonylethanolamide (AEA)] and 2-arachidonoylglycerol (2-AG), has been shown to control feeding in both animals and humans. Indeed, both exogenous and endogenous cannabinoids stimulate food intake through several mechanisms [2]. Conversely, CB1 receptor blockade suppresses food intake and genetically engineered mice lacking the CB 1 receptor eat less after food deprivation [3] and are leaner and less susceptible to developing diet-induced obesity than their normal littermates [4].

The body absorbs 10-25% of the THC when it is inhaled and only 6% when it is eaten. A week after smoking cannabis only 60-70% of the THC has left the body because it is stored in the body fat. It takes about 4-6 weeks before THC is undetectable in the blood. On the other hand, the psychoactive effects are gone after a couple of hours. THC or decomposition products can be detected in urine for a couple of days after the last use. In the urine of chronic users it can even be detected for a couple of weeks after the last use [5].

The mass of body fat is now known to be regulated by several hormones and neuropeptides. Two of these, the circulating peptide hormones leptin and ghrelin have actions that include reciprocal effects on appetite-regulating neurons in the hypothalamus [6]. Ghrelin is an endogenous ligand for the growth hormone secretagogue (GHS) receptor. This peptide was isolated from the stomach and also produced in the hypothalamic arcuate.
nucleus in humans. It is characterized by the presence of an acylated group representing a new type of molecular hormonal structure; it is able to stimulate GH secretion and able to induce adiposity by activating a central mechanism for increasing food intake and decreasing fat utilization [7].

In a number of species, including man, the administration of exogenous and endogenous cannabinoids leads to robust increases in food intake and can promote body weight gain. These effects are believed to be mediated through activation of the CB1 receptor [8]. Also, Kirkham [9] and Rodondi [10] reported that, Marijuana use has been associated with increased appetite, high caloric diet, acute increase in blood pressure and decreases in high-density lipoprotein cholesterol and triglycerides. Recreational users of cannabis report increased appetite and often do eat more, but through which pathway this process is mediated is not exactly known [11,12].

So the aim of this study is to examine the effect of smoked cannabis on the ghrelin plasma concentration and to find its relation to obesity. Also to investigate the possible interactions between ghrelin and endocannabinoid levels in relation to appetite.

Subjects and Methods

Following approval of the Hospital Ethics Committee, written informed consent was obtained from all patients. This study was carried out on 107 male subjects. All participants were between 29 and 52 years of age, non-diabetic and healthy according to a physical examination and routine laboratory tests. 63 patients (cannabis users) were admitted or coming for toxic screening in NECTR (National Environmental Center for Toxicological Research), Kasr El Aini Hospital, Cairo University joined this study. They were found to be positive only for cannabis in urine examination (>50ng/ml), then they were instructed not to use any form of cannabis for 4 weeks and to come again for recheck for cannabis level in urine. The patients were not consecutive, but were selected according to their body mass index (BMI). Obesity was defined as BMI >30kg/m², according to the criteria of both World Health Organization and the International Obesity Task Force.

The patients were grouped into:

- Group 1: 34 obese persons with BMI >30kg/m².
- Group 2: 29 non-obese persons with BMI <30kg/m².

Beside 44 healthy persons found to be negative for cannabis in urine were selected as a control group and they were selected and grouped according to their BMI to:

- Group 3: 23 obese persons with BMI >30kg/m².
- Group 4: 21 non-obese persons with BMI <30kg/m².

Samples collection:

1- Blood: 10ml of venous blood samples were collected from the cannabis smoking group in "first and second visits" and the control group after an over night fast from all subjects joining this study. The samples were immediately cooled to 4°C and centrifuged at 3,000 rpm for 10 minutes at 4°C. Plasma was stored at −20°C until assay.

2- Urine: Second morning urine samples were collected from each subject and from patients in the first and the second visits, in a sterile plastic urine container. All subjects washed their hands with soap and water prior to sample collection to avoid contamination.

All persons were subjected to the following:

1- Complete history taking with special stress on the appetite state and the frequency of meals.

2- Complete medical examinations, body weight and stature were measured to the nearest 0.1kg and 0.1cm, respectively. The BMI was calculated as follows: BMI= Weight/Height m² [13].

3- Plasma glucose level was estimated by God-PAP enzymatic colorimetric method, normal values = 70-110mg/dL [14].

4- Human plasma ghrelin was measured in a fasting state by commercially available radioimmunoassay (Phoenix pharmaceuticals, Belmont, CA) [15].

5- Urinary cannabis level (Δ9-tetrahydrocannabinol) was estimated by the Emit II plus Cannabinoid Assay, which is a homogenous enzyme immunoassay technique used for the analysis of specific compound in human urine (cannabinoid) [16].

6- Serum total cholesterol level was measured by quantitative colorimetric method at 340nm and 37°C (Cat. No. 94545, BioAssay Systems, EnzyChromTM, USA) [17].

7- Serum triglyceride level was quantified by colorimetric method (spectrophotometry at=570 nm) (Cat. No. K622-100, Triglyceride Assay Kit, BioVision) [18].
Statistical analysis:

Statistical analysis was performed using computer statistical software package SPSS 9.02. Descriptive statistics was presented as mean ± standard deviation. Comparative analysis between different groups was applied using student’s t test for parametric data and Wilcox on sum of rank for skewed data. To study the relationship between two quantitative variables Pearson’s correlation coefficient (r) was calculated. p-value is considered significant if <0.05.

Results

The appetite state among the cannabis smokers (groups 1 & 2) was increased compared to the control group as evident by increased frequency of meals per day. Fasting plasma ghrelin concentration was statistical insignificantly decreased in obese subjects (groups 1 & 3) compared to the non obese subjects (groups 2 & 4). While plasma ghrelin concentration was significantly increased (p<0.05) in all patients smoking cannabis (groups 1 & 2) compared to the control subjects (groups 3 & 4). All subjects were non diabetic as evident by normal blood glucose levels. The urinary Δ9-THC level was statistical significantly increased (p<0.05) in the cannabis smokers (groups 1 & 2) compared to control groups (groups 3 & 4) as shown in Table (1). The obese subjects (groups 1 & 3) showed significant increase in the serum levels of cholesterol and triglyceride compared to the non-obese subjects (groups 2 & 4) and there were no correlation between their serum levels and serum ghrelin level.

Table (1): Demographic and biochemical characteristics of the study population (Data represented as mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>Group 1 Obese</th>
<th>Group 2 Non obese</th>
<th>Group 3 Obese</th>
<th>Group 4 Non obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>34</td>
<td>29</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td>Age (years)</td>
<td>27.5±4.5</td>
<td>29.1±4.3</td>
<td>27.1±6.2</td>
<td>28.5±4.9</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>32.36±1.46</td>
<td>26.36±0.67</td>
<td>32.9±1.41</td>
<td>25.8±2.1</td>
</tr>
<tr>
<td>Meals/day</td>
<td>9*</td>
<td>8*</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Ghrelin (pg/ml)</td>
<td>122.56±9.9*</td>
<td>133.63±8.91*</td>
<td>98.6±9.64</td>
<td>102.35±9.4</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>91.0±7.3</td>
<td>84.5±9.5</td>
<td>90.5±8.2</td>
<td>86.5±8.5</td>
</tr>
<tr>
<td>Δ9-THC (ng/ml)</td>
<td>110±5.4*</td>
<td>95.6±6.3*</td>
<td>10±1.2</td>
<td>8±1.6</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>420±6.5†</td>
<td>145±3.4</td>
<td>364±4.7†</td>
<td>136±3.9</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>365±5</td>
<td>124±2.6</td>
<td>296±4.5</td>
<td>95±1.4</td>
</tr>
</tbody>
</table>

* → Significant p<0.05 comparing to control group.
† → Significant p<0.05 comparing to the non obese groups.

Table (2): Comparison between plasma ghrelin (pg/ml) and Δ9-THC (ng/ml) levels in studied groups and control groups during 1st visit and 2nd visit.

<table>
<thead>
<tr>
<th></th>
<th>Ghrelin (pg/ml)</th>
<th>Δ9-THC (ng/ml)</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr.1 1st visit</td>
<td>122.56±9.91</td>
<td>105±5.4</td>
<td>*&lt;0.05</td>
<td>&gt;0.05</td>
<td>*&lt;0.05</td>
</tr>
<tr>
<td>Gr.1 2nd visit</td>
<td>102.3±7.33</td>
<td>25.5±1.4</td>
<td>*&lt;0.05</td>
<td>&gt;0.05</td>
<td>*&lt;0.05</td>
</tr>
<tr>
<td>Gr. 3 control</td>
<td>98.6±9.64</td>
<td>10±3.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gr.2 1st visit</td>
<td>133.63±8.91</td>
<td>95±6.3</td>
<td>†&lt;0.05</td>
<td>&gt;0.05</td>
<td>†&lt;0.05</td>
</tr>
<tr>
<td>Gr.2 2nd visit</td>
<td>117.3±9.84</td>
<td>16.6±3.1</td>
<td>†&lt;0.05</td>
<td>&gt;0.05</td>
<td>†&lt;0.05</td>
</tr>
<tr>
<td>Gr. 4 control</td>
<td>102.35±9.4</td>
<td>8±1.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significance of plasma ghrelin, † Significance of urinary 9-THC (ng/ml).

Table (2) showed that, when the patients stopped smoking cannabis for 4 weeks the mean plasma ghrelin level in groups (1 & 2) in the first visit (122.56±9.91Pg/ml and 133.63±8.91Pg/ml respectively) were significantly decreased in the second visit (102.3±7.33Pg/ml and 117.3±9.84Pg/ml respectively). Also, there is statistical significant difference (p<0.05) between the plasma ghrelin levels in group 1 in the first visit and group 3. There is no statistical significant difference (p>0.05) between plasma ghrelin level of group 1 in the second visit and group 3.
There is statistically significant difference between the plasma ghrelin levels in the first and the second visit ($p<0.05$) in group 2. Also, there is a statistical significant difference between the plasma ghrelin levels in group 2 in the first visit and group 4 ($p<0.05$). There is no significant statistical difference ($p>0.05$) in the mean plasma ghrelin levels of group 2 in the second visit and group 4.

Plasma ghrelin (pg/mL) concentration was negatively correlated with BMI ($\text{kg/m}^2$) in groups (1 & 2) with correlation coefficient $r=-0.8549 & -0.81177$ respectively. Also, plasma ghrelin concentration (pg/mL) was positively correlated with the A9-THC urine level in the first visit in both group 1 and group 2 with correlation coefficient $r=0.9150 & 0.8889$ respectively.

**Discussion**

Obesity and addiction are serious national health problems that may have much in common. The results of the present study demonstrated that, the appetite state of the cannabis smokers (groups 1 & 2) was increased significantly as compared to the control group. Also, the frequency of feeding was increased in groups 1 & 2 as evident by increased number of meals that a person eats each day. These findings were in agreement with several reports which demonstrated that administration of cannabinoi stimulates food intake in animal models. Both peripheral and central administration of anandamide (form of cannabis), increase food intake in rodents [2,19,20]. Also, a study done by Haney et al. [11] who reported that, smoked active marijuana significantly increased total daily caloric intake by 40%. Increased food intake was evident during both private and social periods. The increase in caloric intake was due to an increased consumption of snack foods as a consequence of an increase in the number of snacking occasions. There was no significant change in caloric consumption during meals. The principal increase within the category of snack foods was in the intake of sweet solid items, e.g., candy bars, compared to sweet fluid, e.g., soda, or savory solid items, e.g., potato chips. Increases in body weight during periods of active marijuana smoking were greater than predicted by caloric intake alone. Stimulation of the cannabinoid receptor (CB1) with endocannabinoids such as anandamide could induce an increase in food intake leading to body weight gain [21].

As regards to BMI among cannabis smokers the results revealed that, fasting plasma ghrelin concentration was negatively correlated with body mass index, these result was in agreement with Shiiya [22], Wren [23] and Cummings et al. [24]. Also, Monti et al. [25] evaluated the relationship of ghrelin and leptin hormones with body mass index (BMI) and waist circumference in a population-based random sample of adult men and women subsequently categorized from normal weight to severely obese based on BMI criteria. They found that, total ghrelin was inversely associated with BMI and waist circumference.

Fasting plasma ghrelin concentrations were lower in obese subjects than the non obese subjects, whether they were cannabis smokers or not, these results were in agreement with Tschöp et al. [26] who reported that, the obese subjects have lower plasma concentrations of the adipogenic hormone ghrelin than age-matched lean control subjects. These data seem to indicate that ghrelin is down-regulated in human obesity. Also, they propose that the decreased plasma ghrelin concentrations observed in obesity represent a physiological adaptation to the positive energy balance associated with obesity [27]. Liu et al. [28] explored the effects of expression and secretion of ghrelin on high-fat diet induced obesity in rats. They found both fasting plasma ghrelin concentration and the preghrelin expression in gastric tissue were significantly lower in high-fat diet induced obesity group than in dietary induced obesity resistant and control group. They conclude that lower expression and secretion of ghrelin were closely associated with high-fat diet induced obesity and their higher caloric intake. Also Daghestani et al. [29] studied the relationship that exists between leptin, ghrelin, insulin, neuropeptide Y (NPY), anthropometric, and metabolic variables in Saudi females. They found that, ghrelin concentration decreased in obese and overweight subjects compared to lean subjects. Ghrelin levels were negatively correlated with BMI in obese, overweight and lean subjects. The orexigenic hormone ghrelin induces weight gain by stimulating food intake. Ghrelin concentration was decreased despite the presence of hypertension in the patients who had BMI above 35kg/m$^2$ [30].

The results of the present study demonstrate that, persons positive to cannabinoid intake (A9-THC >50ng/ml) had statistically significant higher plasma ghrelin levels when compared with the negative persons (A9-THC <50ng/ml), while there was no significant difference as regards their BMI. This finding suggests that cannabis intake increases ghrelin secretion which is an orexigenic (appetite stimulant) hormone, also this may explain the appetite-stimulating effect of cannabis. These results were in agreement with Fride et al. [19] reported that THC, or A9-tetrahydrocannabinol,
the major active component of the marijuana plant, stimulates eating in people. The drug significantly increased food intake. Substantial increases in daily caloric intake are routinely observed after cannabis smoke inhalation, primarily through an increase in the frequency and consumption of snack foods, such as candy bars, cookies [31].

Fride [12] found that, smoking cannabis (2.0 or 3.9 percent THC) four times daily "produced substantial increases in food intake with little evidence of discomfort and no impairment of cognitive performance". On average, patients who smoked higher-grade cannabis (3.9%) increased their body weight by 1.1 kg over a four-day period. Researchers reported that inhaling cannabis increased the number of times subjects ate during the study, but did not alter the average number of calories consumed during each meal.

There was a statistically significant positive correlation between plasma ghrelin concentrations and the A9-THC urine level in the first visit in all patients (obese and non-obese), which may explain the state of increased appetite found in cannabis smokers. Tucci et al. [32] reported a significant interaction between the ghrelin and endocannabinoids. Also, study done by Cani et al. [33] suggested that their short-term action on appetite which seems to be in accordance with the control of secretion of ghrelin.

The results of this study provided an evidence for a functional relationship between urinary cannabinoid levels and plasma ghrelin concentration in the increased appetite among cannabis smokers and their relation to the obesity. Also, ghrelin can be considered as a potentially important peripheral signal to the brain stimulating food intake in cannabis smoking and a hormone which mediates the orexigenic effect of cannabis and its relation to obesity, also our findings consolidate existing evidence for the importance of this hormone in the regulation of appetite among cannabis smoking.

References

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