Fasting leptin and acyl ghrelin levels in obese and lean female

Etika Ratna Noer¹, Martha Ardiaria¹, Luthfia Dewi², Darmawati Ayu Indraswari³, Mohammad Sulchan¹, Alifia Mukti Fajrani¹

¹Department of Nutrition, Universitas Diponegoro, Semarang, Indonesia
²Department of Nutrition, University of Muhammadiyah Semarang, Indonesia
³Department of Physiology, Universitas Diponegoro, Semarang, Indonesia

*Corresponding author:
Etika Ratna Noer
Department of Nutrition, Universitas Diponegoro, Tembalang
Semarang, Indonesia
E-mail address: etikaratna@fk.undip.ac.id

Abstract
Background: It was understood that obese individuals had poor control of intake. Further, leptin and acyl ghrelin (AG) are two key hormones that influence the feeling of satiety and hunger. However, appetite hormone pathways with varying nutritional status have not been thoroughly studied.

Aims: In this study, we investigated the correlation between the nutritional status in obese and lean females with plasma levels of leptin and AG.

Settings and Design: A cross-sectional study on 16 obese and 16 lean females was performed.

Methods and Material: We examined 32 female adolescents aged 19–20 years: 16 with normal body mass index (BMI) and 16 with BMI ≥25 kg/m². Height was measured by stadiometer to the nearest 0.1 cm, and body weight and BMI were recorded using bioelectrical impedance analysis. Then, a blood sample was obtained after a night of fasting (10 hrs). Fasting plasma leptin and AG have been calculated using an enzyme-linked immunosorbent assay.

Statistical analysis used: The statistical software program we used to evaluate data with p value < 0.05 was considered statistically significant.

Results: In this study, both leptin and AG were found to be higher in obese. There is a strong association between BMI and leptin (r= 0.929, p= 0.001) suggesting an increase in leptin and adiposity. Furthermore, the positive correlation between BMI and AG (r= 0.904, p= 0.001) showed that the hunger states were obese. Meanwhile, there was a negative correlation between L/G ratio and BMI (r= −0.603, p= 0.001) which indicated an increase in hunger state when the BMI increased.

Conclusion: Leptin resistance and increased AG in obese subjects might be involved in uncontrolled eating behavior.

Keywords: leptin, acyl ghrelin, obese female
Key Messages:
Ghrelin and leptin are hormones that regulate appetite. In obese, the amount of AG after eating is considered to be higher which triggered hunger feeling more quickly. Higher AG levels and leptin were found to be higher, meanwhile the L/G ratio were higher in lean females.


Introduction
Obese people are known to have coordination issues between the brain, digestive tract, and adipose tissue (1). However, several hormones and nutrients help this communication process. The nutritional composition has an effect on the function of the appetite hormone (2). Ghrelin and leptin are hormones that regulate appetite. Obese people found a problem, i.e., the amount of AG after eating is considered to be higher than normal people which triggered hunger feelings more quickly. Abdominal obesity subjects were unable to monitor their consumption of food because they did not feel full (3). Although leptin sends a signal of satiety to the brain to stop eating, obese subjects undergo leptin resistance because leptin receptors are disrupted, resulting in failure of leptin signal delivery to the hypothalamus (4). This resistance is associated with increased food consumption due to a dull feeling of fullness, making weight loss difficult to achieve (5).

Considering whether obesity is mainly caused by hyperphagia, the mechanisms for regulating food consumption must be understood to control this chronic disease (6,7). Ghrelin has two different end products: deacylated ghrelin and acylated ghrelin. Acetylated ghrelin (AG) transmits a signal to accurate nucleus neurons resulting in increased appetite and desire to eat (8). Fasting does not increase ghrelin in diet-induced obesity mice, while ghrelin does not decrease as it does in lean subjects in response to meals in obese people (9).

Many obese people exhibit lower ghrelin circulation and blunted meal-related variations compared to lean people. The previous study found that acylated ghrelin was higher in obese than in average-weight subjects. Since obesity has become a global problem, it is important to understand the dynamics of its development and to identify effective prevention and treatment strategies. The objective of the study is to examine the correlation between nutritional status and plasma levels of leptin and AG in obese and lean females.

Subjects and Methods
Ethics statement
This study was reviewed and approved by the Local Ethical Committee of the Faculty of Medicine, Diponegoro University, and Dr. Kariadi Hospital, Semarang, Central Java, Indonesia (427/EC/FK-RSDK/VII/2018). All participants issued informed written consent. Samples that met the study criteria were collected using consecutive sampling.
Participants

Thirty-two participants of this current study were obese women from Semarang, Central Java, Indonesia. The eligibility of participants was based on the following inclusion criteria: (1) 25–45 years of age, (2) BMI > 25 kg/m² and hip waist > 85 cm for obese women, (3) healthy, free from severe metabolic, cardiovascular, or endocrine diseases, and (4) not using any oral contraceptive pills and taking drugs at the time of the study. The main method of recruitment of participants for this study was the use of posters which were distributed across public facilities (offices and hospitals) in the city of Diponegoro National Hospital. All participants provided informed consent prior to participation. In addition, anthropometric, body composition, and biochemical measurements were conducted after a 12-h fasting period.

Anthropometric and body composition measurements

With the participants standing without shoes, height was measured and recorded to the nearest half centimeter. Moreover, weight and body fat was measured with the participants wearing light clothing or underwear (nearest 0.5 kg). Height (cm) and weight (nearest 0.1 kg) measurements were then obtained, and body mass index (BMI), defined as weight divided by height squared (kg/m²), was also calculated. The waist circumference (cm) was calculated as the horizontal distance around the abdomen at the umbilical level, while the hip circumference (cm) was calculated as the largest circumference between the waist and thighs. Height, waist, and hip measurements were recorded to the nearest 0.1 cm. Percent body fat (% BF) and visceral fat were measured using Tanita BIA (Tanita, Amsterdam, EU).

Lifestyle assessment

Participants completed a self-administered screening questionnaire, including smoking (smoker, non-smoker) and medication (medication user, non-medication user) and a series of questionnaires to collect information regarding lifestyle, dietary habits, and physical activity.

Biochemical measurements

Blood samples were taken into EDTA tubes in the morning following a 12-h night fasting and were then cold centrifuged (2800 rpm) for 15 min, while plasma samples, assayed in duplicate, were stored in cryo-microtubes at −80 °C. Plasmaleptin and AG concentrations were determined using an enzyme-linked immunosorbent assay. Intra- and inter-assay coefficients of variation are less than 3.3 and 7.6 %, respectively, and the sensitivity, measured by interpolation, is 9.5 pg/ml.

Statistical analysis

Unless otherwise specified, data are presented as means ± standard deviation (SD). Correlations of leptin, AG, and L/G ratio with BMI according to age were analyzed using Pearson’s correlation. Further, all statistical analyses were performed using SPSS.
Results

Subject Characteristics

A total of 32 young adult females participated in this study. Baseline characteristics are shown in Table 1. As can be seen, there were significant differences between the characteristics of the groups, excluding the height, and AG levels in obese were higher.

Table 1. Baseline characteristics of the participants

<table>
<thead>
<tr>
<th>Variables</th>
<th>Obese n=16</th>
<th>Lean n=16</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>19.17 ± 0.98</td>
<td>19.87 ± 0.92</td>
<td>0.005</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>156.87 ± 4.63</td>
<td>155.69 ± 4.59</td>
<td>0.921</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.84 ± 8.77</td>
<td>51.28 ± 4.17</td>
<td>0.007</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.45 ± 3.01</td>
<td>21.15 ± 1.25</td>
<td>0.001</td>
</tr>
<tr>
<td>Leptin (pg/mL)</td>
<td>48.69 ± 7.11</td>
<td>24.67±3.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Acyl ghrelin (pg/mL)</td>
<td>43.4±12.14</td>
<td>15.03±2.22</td>
<td>0.001</td>
</tr>
<tr>
<td>Ratio L/G</td>
<td>0.12±0.03</td>
<td>0.17±0.04</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD

Leptin and AG levels in obese and lean female

The mean plasma concentration of AG in the obese group (OG) was 43.4±12.14 pg/mL and was significantly higher compared to the non-obese group (NO) (15.03±2.22 pg/mL), differed significantly with p = 0.001. On the other hand, leptin plasma concentration in OG (48.69 ± 7.11 ng/ml) was significantly higher than in NO (24.67±3.3 ng/ml), differed significantly with p = 0.001.

Ratio Leptin to Ghrelin in obese and lean female

Subsequently, the L/G ratio was compared between normal body weight and overweight/obese subjects and was later on found to be lower in OG compared to NO with mean 0.12±0.03 and 0.17±0.04, respectively, differed significantly with p = 0.001.

Table 2. Correlations with BMI between leptin, AG, and L/G ratio

<table>
<thead>
<tr>
<th>Variables</th>
<th>p</th>
<th>r</th>
<th>p</th>
<th>r</th>
<th>p</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.0001</td>
<td>0.929</td>
<td>0.0001</td>
<td>0.904</td>
<td>0.0001</td>
<td>-0.603</td>
</tr>
</tbody>
</table>

Data determined by Pearson correlation; * P values are significant at<0.01

Analysis of correlation with BMI between leptin, AG, and L/G ratio in obese groups

Analysis of correlations with BMI between leptin, AG, and L/G ratio as shown in Table 2 demonstrated that ghrelin in OG was statistically significant with BMI with p = 0.001 and r = 0.904 (Figure 1). Similarly, leptin was positively correlated with BMI, and these correlations were statistically relevant with p = 0.001 and r = 0.929 (Figure 2). A positive correlation was observed between the BMI and L/G ratio with p = 0.001 and r = 0.929 (Table 2).
Discussion

The study showed that higher AG levels and L/G ratio were found to be higher in obese females compared to average-weight subjects. Furthermore, regulation of serum acylated ghrelin levels appears to be tightly regulated by leptin in this study (10). Changes in the direction of leptin effect on acylated ghrelin levels from positive in lean women to obese women were observed. The opposite effects of ghrelin and leptin on energy balance and appetite control has long been identified.
at the hypothalamic level, but there is an increasing evidence that both hormones may function peripherally on the regulation of various metabolic processes, such as glucose metabolism (11). This is in line with the observation that acylated ghrelin (but not desacyl form) may increase glucose from primary hepatocytes (12) and that leptin may increase glucose turnover and glucose uptake by peripheral tissues (13). It can therefore mean that leptin indirectly increases glucose as a hepatic output by inducing acylated ghrelin secretion. In this study, both hormones were assessed under basal conditions to resolve this problem. Later on, the results showed a positive linear association between acylated ghrelin and leptin in obese females.

Ghrelin concentrations in the blood are lower in obesity and obesity-associated type 2 diabetes (T2D). However, under these pathophysiological conditions, the types of ghrelin may differ and are currently controversial (14). Previous studies have shown that ghrelin levels were higher in lean individuals than in overweight and obese individuals, but our study showed otherwise. This can be explained by the fact that ghrelin may help maintain body weight and BMI in healthy individuals. The hormonal mechanism of ghrelin might not work in obesity, as in many other hormones (15). This is further demonstrated by the disparity in the correlation seen in obese individuals; it is known that ghrelin levels are positively correlated with BMI. Controversy over the results of research on BMI and ghrelin levels is still ongoing. Moreover, several previous studies supported the increase in AG levels after weight loss (16). This discrepancy occurred due to the form of ghrelin examined (active ghrelin and total ghrelin) and the rigid test process, so the findings did not match any similarities. Most studies measure total ghrelin levels. According to recent studies, acylated ghrelin has a biological potential (17).

The L/G ratio was significantly increased among obese females (18). We decided to examine leptin and ghrelin together as L/G ratio, since the regulation of the metabolic state of the body, appetite, and hunger states, etc., is more likely controlled by the interaction of these two hormones than by the action of any individual hormone (13). As a result, a higher L/G ratio could have more beneficial effects in subduing the feeling of hunger (18). However, there is no publication on either the relationship between the L/G ratio and the control of hunger and satiety, or the metabolic states and consequences (19). Previous studies have shown that there was a correlation between increased L/G ratio and increased hunger and appetite (10); thus, it is proposed that with a higher L/G ratio, hunger and appetite could be decreased.

The limitation of this study involves limited sample size and female-only enrolment. All-female subjects were to reduce the impact of potential mitigating factors, such as the effects of sex hormones and sex differences, or the time spent on digestion and absorption of food or on each visit, etc. These factors may, however, interfere with our results. The study adds valuable knowledge that leads to the ability to differentiate between cases and control of obese women despite limitations. In conclusion, leptin, AG, and L/G ratio are positively correlated with BMI in obese women. And thus, increased leptin and AG secretion in obese subjects could be involved in unregulated eating behavior.
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References


