PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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OPEN ACCESS Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2017.38.44

Research Article
Effect of International and Indonesian Fast Foods on Plasma Ghrelin and Glucagon Like Peptide-1 (GLP-1) Levels, Hunger and Satiety Scores is Similar in Obese Adults

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Abstract

Background and Objective: The desire for food is regulated by appetite-associated hormones such as ghrelin and glucagon-like peptide-1 (GLP-1). Hormone release is influenced by the energy density of and macro-nutrients in food. This study assessed the effects of international and Indonesian fast food on ghrelin and GLP-1 levels, which contribute to the perception of hunger and satiety, in obese individuals. **Materials and Methods:** A total of 16 obese individuals were provided international fast food; after a week, the subjects were provided Indonesian fast food. Plasma samples were collected and the perceptions of hunger and satiety were assessed using a visual analogue scale before consuming time 0, 30, 60 and 120 min after consuming both kinds of fast food. Data were analyzed using unpaired t-tests, Mann-Whitney U tests, Pearson's correlation coefficients and Spearman's rank correlation coefficients. **Results:** The results revealed significant decreases in plasma ghrelin levels after 120 min of eating international and Indonesian fast foods. In contrast, plasma glucagon-like peptide-1 hormone levels did not differ significantly after consuming both kinds of fast food. The results also showed no correlation between changes in the plasma levels of ghrelin and GLP-1 and the perceptions of hunger and satiety in obese individuals. **Conclusion:** It is concluded that both types of fast food resulted in equal suppression of hunger and increased satiety.

Key words: International fast food, Indonesian fast food, ghrelin, GLP-1, VAS, obesity

Received: September 20, 2016 Accepted: November 04, 2016 Published: December 15, 2016

Citation: D. Handayani, L.D.A. Oktafiani, R.T. Abednego and S. Andarini, 2017. Effect of international and Indonesian fast foods on plasma ghrelin and Glucagon Like Peptide-1 (GLP-1) levels, hunger and satiety scores is similar in obese adults. Pak. J. Nutr., 16: 38-44.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Obesity is a serious global problem and is also prevalent in Indonesia. The prevalences of obesity among children, adolescents and adults in Indonesia are 8.8, 1.6 and 15.4%, respectively. Moreover, the prevalence of obesity has recently increased[1]. Various factors, including energy imbalance, dietary changes and an unhealthy lifestyle may precipitate obesity[1,2].

Obesity is a chronic disease related to the excess accumulation of fat in adipose tissue[3]. This accumulation increases the production of leptin, an important biochemical regulator of hunger and satiety and ultimately energy balance. However, constant leptin production can cause leptin resistance[4]. Obesity is a major risk factor for metabolic diseases such as diabetes mellitus, cardiovascular disease, hypertension, dyslipidemia and cancer[2].

Obesity is exacerbated by the societal trend of consuming fast foods, as they are easy to prepare, carry and consume and are considered practical, fashionable, delicious and attractive[5,6]. Fast food is defined as food with a high energy density due to high calorie and fat content, which can lead to excess energy consumption[5,7,8].

Previous study revealed that international fast food in Indonesia tends to have a higher energy density and fat content than Indonesian fast food[9]. Because Indonesian people tend to choose international fast food, the incidence of obesity is increasing and becoming a problem[1]. In addition, there is a lack of information on the effect of fast food on the satiety response, particularly among obese individuals.

Appetite regulation in the body is controlled by gastrointestinal hormones; i.e., ghrelin and glucagon-like peptide-1 (GLP-1). These hormones play important roles in regulation and indication of hunger and satiety. Ghrelin and GLP-1 secretion is stimulated by food intake[2,10]. In addition to measurement using hormone levels, satiety can be measured using a Visual Analogue Scale (VAS), which is a subjective method to assess hunger, satiety or fullness and likeliness to eat[11]. The present study assessed whether there were differences in satiety as measured by ghrelin and GLP-1 levels and a VAS and investigated the relationship between changes in hormone levels and VAS scores after consuming international and Indonesian fast foods.

MATERIALS AND METHODS

Subjects: The respondents were 16 obese male adults aged 19-29 years with body mass indexes (BMIs) >27.5 kg m^{-2}. All respondents were declared healthy based on screening and did not have hypercholesterolemia, diabetes mellitus or hypertension. This study was approved by the Research Ethics Committee of Faculty of Medicine, University of Brawijaya, Indonesia (No. 389/EC/KEPK/07/2015).

Research design: This crossover study analyzed the relationship between satiety hormones and VAS after consumption of international and Indonesian fast foods. All respondents received two fast food "Treatments" at different times, with an internal of 1 week. Each respondent consumed international and Indonesian fast foods with the same energy content at breakfast. At 9 pm the previous night, the respondents consumed a dinner consisting of 25% of the respondent's daily requirements. The respondents then fasted for 10-12 h. Then, at 7 am, blood was collected and the respondents completed the VAS (time 0). Next, the respondents at the international fast food. Then, blood was collected again and the respondents completed the VAS 30, 60 and 120 min after eating. One week later, the respondents were provided Indonesian fast food and underwent the same blood sampling and VAS procedures.

Fast foods: The fast foods were obtained from restaurants located in Malang. They included both international and Indonesian fast foods. The international fast foods consisted of black pepper chicken fettuccine (Pizza Hut), fried chicken and rice (Kentucky Fried Chicken), fried chicken with mayonnaise and salad (Kentucky Fried Chicken) and a double burger with french fries (McDonalds). The Indonesian fast foods included chicken soup (Sop Ayam Pak Min Klaten, an Indonesian restaurant located in the City of Malang), meatballs (Bakso Presiden, an Indonesian restaurant located in the City of Malang), black curry (Rawon) (Rawon Bu Gito, an Indonesian restaurant located in the City of Malang) and chicken satay (Sate Ayam Pak SIboen, an Indonesian restaurant located in the City of Malang. These foods contained comparable amounts of energy (500-600 kcal). The foods were analyzed using a bomb calorimeter for energy analysis, oven method for water content analysis, difference method for carbohydrate content analysis, Kjedahl method for protein analysis, Soxhlet for fat analysis and enzymatic hydrolysis for fiber analysis.

Blood sampling and biochemical analysis: Plasma samples were obtained from blood samples that were centrifuged at 3,000 rpm and incubated at 25°C for 10 min. The PLC-05 tabletop centrifuge (Gammy Industrial Corporation, Taiwan in association with Cannic, Inc., USA) was used. Satiety (ghrelin and GLP-1 levels) was measured using a sandwich

enzyme-linked immunosorbent assay (ELISA) (E-EL-h2002 and E-EL-H0148; Elabscience, Biotechnology, Beijing). Standard samples (Elabscience, E-EL-h2002 and E-EL-H0148) and plasma samples were placed in a microtiter plate containing specific antibodies (A-GHRL and GLP-1) and incubated at 37°C for 90 min. Then, specific biotinylated detection antibodies and streptavidin HRP-conjugates were added to each microplate and incubated at 37°C for 90 min. The substrate and stop solutions were added in a dark room until the color changed. The optical densities (ODs) were obtained using an ELISA reader at a wavelength of 450±2 nm. Hormone concentrations in the samples were calculated by comparing the OD values against a standard curve.

Appetite and satiety: The respondents were asked to complete a VAS form with parameters of hunger (Q1) and satiety (Q2) at time 0 (before eating) and 30, 60 and 120 min after their meals. The VAS forms were completed by vertically marking a line in accordance with how the respondent felt. In this study, four questions were used to measure appetite and satiety levels. The distance between the extreme left point and the vertical mark on the line was calculated. These results were analyzed using the Satiety Quotient (SQ)[2,12], as follows:

$$\text{Appetite SQ formula} = \frac{\text{Time 0 pre-eating score-120 min post-eating average score}}{\text{Energy content of food intake}} \times 100$$

$$\text{Satiety SQ formula} = \frac{\text{120 min post-eating average score-pre-eating score}}{\text{Energy content of food intake}} \times 100$$

Statistical analysis: The differences among the international and Indonesian fast foods and ghrelin plasma levels were tested using unpaired t-tests (data were distributed normally and homogeneous). Meanwhile, GLP-1 plasma levels and VAS scores (Q1 and Q2) were tested using Mann-Whitney tests (data were not normally distributed and homogeneous). The correlations between ghrelin levels and VAS scores (Q1) were assessed using Pearson's tests (data were distributed normally and homogeneous). The correlations between GLP-1 levels and VAS scores (Q2) were assessed using Spearman's tests (data were not normally distributed and homogeneous). These statistical analyses were performed using IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, NY, USA).

RESULTS

Respondent characteristics: The respondents were aged 19-27 years. Their average weight and height were 97.0±17.9 kg and 169.5±5.5 cm, respectively. The BMIs ranged from 28.3-45.2 kg m^{-2}. The average blood glucose and cholesterol levels were 112.2±21.1 and 127.0±43.1 g dL^{-1}, respectively. The measurements and anthropometric characteristics of the respondents are shown in Table 1.

Fast food characteristics: Eight foods (four international and four Indonesian fast foods) were used in this study. Both types of foods were used and tested using unpaired t-tests. In Table 2, the results of the analysis showed no significant differences in content by weight per serving, energy, energy density, carbohydrate, protein, fat and fiber between both types of fast foods, p = 0.069, 0.872, 0.098, 0.412, 0.366, 0.112 and 0.587, respectively.

Gastrointestinal hormones: Changes in gastrointestinal peptide levels with both groups of fast foods were analyzed using Mann-Whitney and unpaired t-tests. Handayani *et al.*[13] described changes in plasma levels of ghrelin hormone, which were tested using unpaired t-tests. These levels differed significantly between international and Indonesian fast foods at 120 min (-1468.75±1195.21 vs -132.50±958.20 pg mL^{-1}, p = 0.02)[13] (Fig. 1).

Meanwhile, changes in GLP-1 levels were analyzed using Mann-Whitney tests, with no significant differences between the two groups of fast foods at each observation time (p = 0.44, 0.067 and 0.522, consecutively starting from 30 min) (Fig. 2).

Appetite level: The VAS values and satiety quotients were analyzed using Mann-Whitney tests. The perception of

Table 1: Respondent characteristics

Characteristic	Mean±SD
Age (years)	21.4±1.9
Body weight (kg)	97.0±17.9
Body height (m)	1.695±0.055
BMI (kg m^{-2})	33.6±4.8
Body fat (%)	30.3±3.6
Waist fat (%)	16.9±4.1
Waist circumference (m)	1.054±0.119
Pelvic circumference (m)	1.136±0.087
Systolic blood pressure (mmHg)	123.7±9.6
Diastolic blood pressure (mmHg)	80.6±4.4
Random blood sugar level (g dL^{-1})	112.2±21.1
Cholesterol level (g dL^{-1})	127.0±43.1
Leptin level (pg mL^{-1})	62.0±21.8

Table 2: Characteristics of fast food

Energy and macro-nutrients	International fast food	Indonesian fast food	Unpaired t-test
Energy (kcal $serving^{-1}$)	559.06	568.24	0.872
Energy density (kcal g^{-1})	2.35	1.11	0.098
Carbohydrate (g $serving^{-1}$)	70.67	84.81	0.412
Protein (g $serving^{-1}$)	18.15	24.74	0.366
Fat (g $serving^{-1}$)	20.62	10.58	0.112
Fiber (g $serving^{-1}$)	30.91	34.17	0.587
Portion weight per serving (g)	264.75	595.25	0.069

Values are means, n = 4

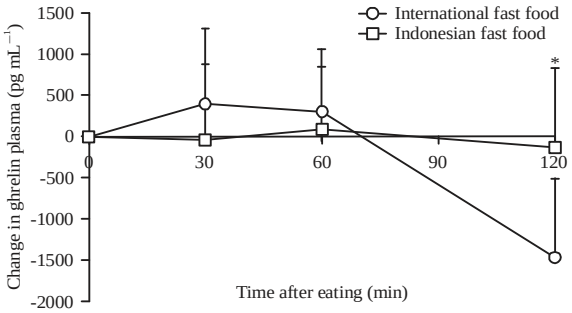

Fig. 1: Changes in ghrelin plasma level after consuming international and Indonesian fast food (significantly different at $p<0.05$)

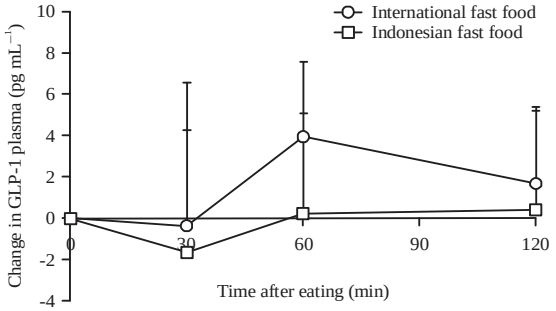

Fig. 2: Changes in GLP-1 plasma levels after eating international fast food and Indonesian fast food

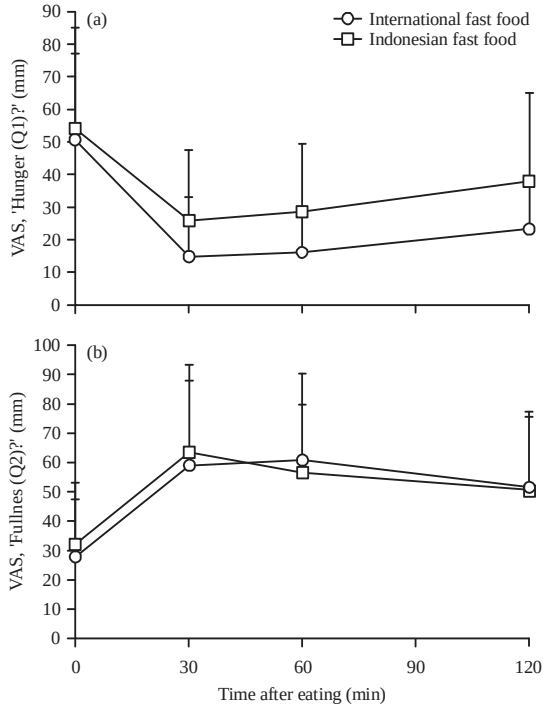

Fig. 3(a-b): VAS score (a) Hunger and (b) Fullness after eating international and Indonesian fast food

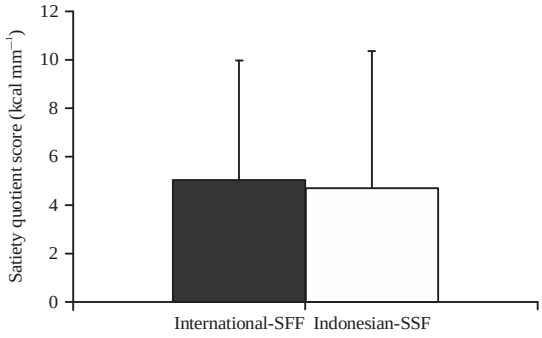

Fig. 4: Average value of satiety quotient international fast food and Indonesian fast food

appetite (Q1) and satiety (Q2) on the VAS did not differ significantly between the fast food groups ($p>0.05$ for all VAS parameters at any observation time) (Fig. 3a-b).

Similarly, there were no significant differences in SQ between the fast food groups ($p = 0.7$) (Fig. 4).

Relationship between gastrointestinal hormone levels, hunger and satiety: The correlations among gastrointestinal hormones and the perceptions of hunger (Q1) and satiety (Q2) were analyzed using Pearson and Spearman statistical tests. There were no significant correlations between plasma ghrelin levels and hunger perception (Q1) before and after eating the international and Indonesian fast foods ($p>0.05$). The relationships between plasma GLP-1 levels and satiety perception (Q2) before and after eating the international and Indonesian fast foods were also not significant ($p>0.05$). However, 60 min after eating the international fast foods, there was a significant and moderate correlation ($r = -0.580$, $p = 0.019$) (Table 3).

DISCUSSION

One intervention to combat obesity is weight loss, achieved by limiting food intake[14]. Food intake is closely associated with gastrointestinal hormone secretion, for which the energy content, energy density and macro-nutrient content play important roles. Therefore, the secretion of gastrointestinal hormones such as ghrelin and GLP-1 can regulate hunger and satiety. Plasma levels of ghrelin, an orexigenic hormone that stimulates hunger will increase before eating and decrease after consuming food. Meanwhile, plasma levels of GLP-1, an anorexigenic hormone, peak after eating, in order to suppress hunger[15,16].

Overall, the results of the current study show that consumption of both types of fast food causes a decrease in

Table 3: Correlation test among gastrointestinal hormone, visual analogue score Q1 and Q2 per time period

Hormone	VAS	Fast foods	Minutes	Significant score (p)	Correlation coefficient (r)
Ghrelin	Q1	International	0th	0.836	-0.056
			30th	0.124	0.400
			60th	0.186	0.348
			120th	0.362	0.244
		Indonesian	0th	0.213	-0.329
			30th	0.986	-0.005
			60th	0.341	0.255
			120th	0.404	0.224
GLP-1	Q2	International	0th	0.621	0.134
			30th	0.297	-0.278
			60th	0.019	-0.580
			120th	0.959	0.014
		Indonesian	0th	0.941	-0.020
			30th	0.181	-0.352
			60th	0.562	0.157
			120th	0.717	0.098

plasma ghrelin levels, with a significant decrease after 120 min consumption of the international fast foods. Plasma ghrelin levels were relatively constant in respondents eating Indonesian fast foods, but the levels increased at 30 min and began to slowly decline until 120 min. These observations are supported by those of Hill *et al.*[17], who found that low energy density does not affect the post-prandial plasma ghrelin levels in obese women. Meanwhile, the consumption of international fast foods caused greater changes in plasma GLP-1 levels than the consumption of Indonesian fast foods at all observation times. Research by Luscombe-Marsh *et al.*[18] indicated that diets high in energy density and fat, low in energy density and high in fat and low in energy density and high in protein do not result in significant changes in glucose and plasma GLP-1 concentrations in overweight and obese individuals.

The energy density of food is an important factor for regulating food intake and satiety, which can control body weight[19]. The energy density of food is dependent on the energy content and weight per portion. The energy content is derived from the macro-nutrients. Fat has the highest energy density, at 9 kcal g^{-1} or twice that of carbohydrates and proteins[2]. In the current study, plasma ghrelin and GLP-1 levels may have been affected by the macro-nutrient density and content in food. Suppression of ghrelin secretion and release of GLP-1 tended to be higher after eating international fast foods containing more fat and greater energy density than those of Indonesian fast foods. The absence of changes in plasma ghrelin and GLP-1 levels was likely due to insignificant differences in energy content, energy density and macro-nutrients between the two types of fast foods. Hence, the variety of foods offered should not have influenced our results.

The VAS measures a subjective response that can be used as a parameter for measuring hunger and satiety, in addition to measuring hormonal levels[11]. In this study, there were no significant differences in the VAS scores for "Hungry" and "Full" after eating the international and Indonesian fast foods. This is likely because the energy density did not differ significantly between the two types of fast foods. Similarly, Karl *et al.*[20] reported no systematic differences in the ratings of hunger and fullness, despite substantial differences in energy intake. Energy density and the portion size independently affect energy intake. The results of the current study indicate that the consumption of international fast foods further suppresses hunger, thereby reducing the desire to eat and reducing food intake. This is in line with the observed lower plasma ghrelin levels and higher GLP-1 levels after eating the international fast foods. In addition, the average SQ was lower for Indonesian fast foods (4.68 mm $kcal^{-1}$) than for international fast foods (5.01 mm $kcal^{-1}$) in the present study. McNeil *et al.*[21] stated that a lower SQ indicates weaker satiety responses to the particular food. Thus, the international fast foods suppressed hunger more than the Indonesian fast foods, although the difference between the two food groups was not statistically significant. Therefore, consumption of international and Indonesian fast foods results in similar levels of satiety.

In the present study, plasma ghrelin levels and the Q1 score did not affect the perception of hunger. This observation is supported by that of Erdmann *et al.*[22], who reported that administration of five kinds of food with different carbohydrate, protein, fat and fiber profiles was not associated with plasma ghrelin levels and hunger and satiety scores. Ghrelin suppression is lacking in obesity conditions, which affects hunger regulation[23].

Chanoine *et al.*[24] also showed that GLP-1 levels do not affect satiety. Several other studies also obtained similar results, which also support the insignificant correlation between plasma GLP-1 concentrations and changes in satiety and hunger among overweight and normal-weight male children. Decreased GLP-1 hormone secretion has been observed in obese people. The mechanism of the reduced GLP-1 hormone secretion in obesity remains unknown but is probably due to leptin resistance and insulin resistance caused by excessive weight gain[10,23]. In the current study, the average leptin level in the obese respondents was 62.0 pg mL^{-1}. This level was significantly higher than that of the normal-weight respondents (18.5-22.9 kg m^{-2}, 21.6 pg mL^{-1}).

Leptin is an important biochemical regulator of hunger and satiety, to promote energy balance. Mutations in the encoding gene of leptin and the resulting changes in transduction signals affect the control of food intake, prompting the onset of obesity[4]. Continuous and excessive production of leptin in obesity triggers leptin resistance. Failed expression of the leptin receptor in the hypothalamus also affects leptin sensitivity. These conditions result in the absence of ghrelin suppression. They also reduce the secretion of GLP-1 and decrease its sensitivity; thus, these hormones can no longer suppress hunger or cause satiety[23,25,26].

CONCLUSION

The lack of significant correlations between the plasma ghrelin and GLP-1 hormone levels, hunger and satiety scores after consuming both types of fast food is likely due to leptin resistance in obese individuals, which changes gastrointestinal hormone levels. Therefore, both types of fast food resulted in equal suppression of hunger and increased satiety.

ACKNOWLEDGMENTS

This study was funded by a Leading Research University Grant by the Ministry of Research, Technology and Higher Education Republic Indonesia 007/Add/SP2H/PL/DIT.LITABMAS/V/2015. In memoriam of Professor M. Rasjad Indra, who is gratefully acknowledged for his valuable discussion of this manuscript.

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