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ORIGINAL ARTICLES

Profiles of peptide YY and ghrelin, levels of hunger and satiety, and *ad libitum* intake in obese and non-obese Indonesian women

FIASTUTI WITJAKSONO[1,*], MARCELLUS SIMADIBRATA[2], WIDJAJA LUKITO[3], ANDI WIJAYA[4], FARIZ NURWIDYA[1]

[1]Department of Nutrition, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital, Jakarta, Indonesia. Address: Jl. Salemba Raya No. 6, Jakarta 10430, Indonesia
[2]Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital, Jakarta, Indonesia. Address: Jl. Salemba Raya No. 6, Jakarta 10430, Indonesia
[3]Southeast Asian Ministers of Education Organization Regional Centre for Food and Nutrition (SEAMEO RECFON), Jakarta, Indonesia. Address: Jl. Salemba Raya No. 6, Jakarta 10430, Indonesia
[4]Department of Clinical Chemistry, Faculty of Medicine Universitas Hasanuddin, Makassar, Indonesia. Address: Jl. Perintis Kemerdekaan KM. 10, Makassar 90245, Indonesia

Introduction. The current study aimed to assess profiles of peptide YY and ghrelin, visual analog scales (VAS) for hunger and satiety, and *ad libitum* intake in obese and non-obese women.

Methods. This open-label non-randomized interventional study involved obese (BMI ≥ 25–35 kg/m^2) and non-obese (BMI 18.5–23.0 kg/m^2) women subjects. Levels of peptide YY and ghrelin were determined by radioimmunoassay and enzyme-linked immunosorbent assay (ELISA), respectively, while the degrees of hunger and satiety were measured using visual analog scale (VAS) questionnaires. The results were compared in fasting condition and in 15, 60, 120, and 180 minutes after breakfast with balance composition formulation. This study also compared the *ad libitum* intake within 4 hours after breakfast.

Results. As compared to the non-obese group, the obese group have significantly lower levels of peptide YY in fasting, and in 15, 60, 120, and 180 minutes post-prandial, and smaller AUC (Area Under the Curve) of fasting peptide YY. Furthermore, the obese group showed significantly higher *ad libitum* intake. The obese group also have lower levels of ghrelin and lower VAS for hunger and higher in VAS for satiety as compared to the non-obese group.

Conclusions. There were significant differences in peptide YY level, 4 hours after breakfast *ad libitum* intake, ghrelin level, and VAS for hunger and satiety, between obese group and non-obese one.

Keywords: peptide YY, ghrelin, hunger, satiation, food intake.

INTRODUCTION

Obesity is a major health problem globally, including in Indonesia. According to the Basic Health Research (Riskesdas) in 2013, the prevalence of obesity based on body mass index (BMI) in Indonesian women above 18 years old was 32.9% [1]. Combination of various risk factors can increase the incidence of obesity, including high calories intake, fat intake, and low physical activity [2]. From a cohort of obese patients who had a diet and lose their weight initially, only 25% will have a sustained weight loss [3]. Hunger and satiety signals were regulated by the hypothalamus and were integrated with the signal of the brain stem [4, 5].

Gastrointestinal tract produces numerous neuropeptides, such as peptide YY, an anorexigenic gut hormone, and ghrelin, an orexigenic gut hormone [6, 7]. Peptide YY is secreted because of the presence of foods in the gut lumen and suppressed appetite [8]. Before a meal, the peptide YY level was low, however, 15 minutes after food enters the mouth, the peptide YY level will increase and reach its peak in 60 minutes after the meal [9]. All macronutrients, mostly fat, will influence the release of peptide YY [10]. Ghrelin is mainly secreted by endocrine cells of the stomach between meals when the stomach is empty and is also secreted during fasting [11]. Studies showed that ghrelin works in cells of the pancreas, hypothalamus, pituitary and a variety of peripheral tissues [12].

Visual analog scale (VAS) is a measurement of subjective sensations of human to have an eating desire or not [13]. As a consistent assessment tool, VAS can describe energy intake and is not influenced by the earlier diet [14–16]. The aim of our study is to assess profiles of peptide YY, ghrelin, VAS for hunger, VAS for satiety, and *ad libitum* intake in obese and non-obese Indonesian women.

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MATERIAL AND METHODS

DESIGN, SETTING, AND SUBJECTS

This open-label non-randomized interventional study had an approval from the Ethics Committee in Faculty of Medicine, Universitas Indonesia. The subjects were invited by the Department of Nutrition, Faculty of Medicine Universitas Indonesia. The inclusion criteria were women aged 20–40 years with body mass index (BMI) 18.5–23 kg/m^2 for non-obese group and 25–30 kg/m^2 for obese group, able to drink milk, had a stable body weight in the last 6 months (change in body weight of 4 kg or less) and had normal blood glucose level. The exclusion criteria were subjects who never had breakfast, were doing a weight loss program, under medications or drugs of fat absorption inhibitor or hunger suppressant, had a history of gastrointestinal resection, were pregnant, were breastfeeding and were refused to sign the informed consent. Subjects who are willing to participate in this study were asked to sign an approval sheet and those who did not complete all research stages would be dropped out.

Before the study started, the subjects should get balanced composition diet in a 3 days period. One day before the study started, they had a fasting period from 10 pm to 8 am and attended in the morning to follow the stages of the study. During the fasting period, the subjects were free to consume water, but they were not allowed to drink in 2 hours before breakfast. Then, the subjects were given a breakfast with balanced macronutrient composition (12.4% protein, 68.2% carbohydrate, and 22.6% fat of energy) in the form of liquid foods or milk material and had the same taste. The total calorie needs were calculated by appropriate calculation of subjects energy needs. Subjects should eat the breakfast within 15 minutes. In that period, they were allowed to consume as much as 600 mL of water within 4 hours.

ASSESSMENT OF OUTCOMES

Peptide YY and ghrelin analysis and VAS measurements for hunger and satiety were performed during fasting period and 15, 60, 120, and 180 minutes after breakfast. The *ad libitum* intakes were compared in 4 hours after breakfast. Peptide YY and ghrelin were determined in accordance with the factory protocols. Peptide YY were analyzed by radioimmunoassay (RIA) of PYY 3-36 fragments using Human PYY 3-36 specific RIA Kit (Millipore-Merck KGaA, Darmstadt, Germany). Ghrelin was determined by using Human Active Ghrelin Elisa Kit (Millipore-Merck KGaA). Hunger and satiety were measured using a visual analog scale (VAS) with 100 mm straight lines, consisting of extreme choices on the two end lines. Subjects choose between the two end lines by giving "X" sign. The flow of study is described in Figure 1.

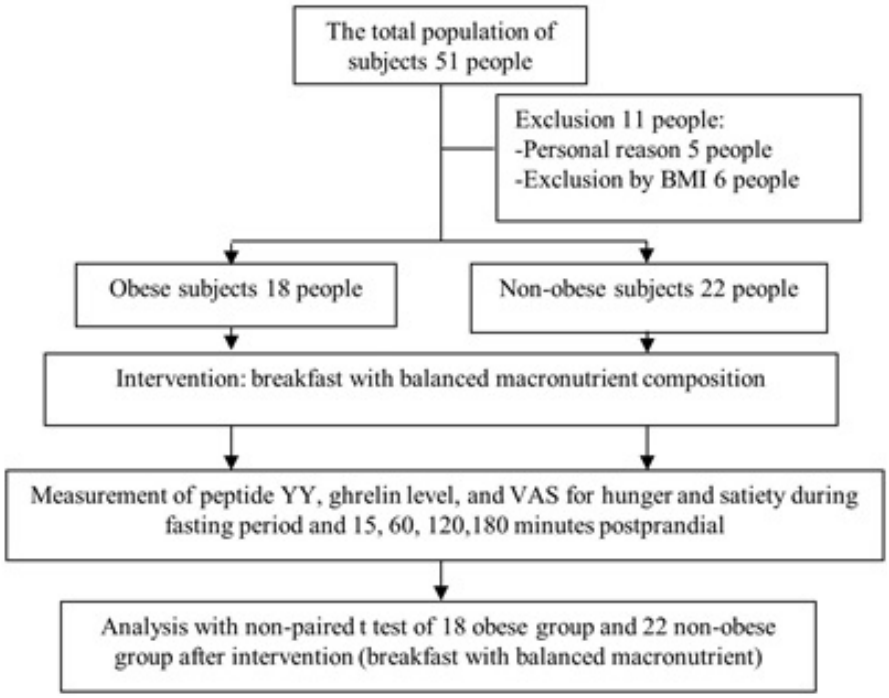

Figure 1. Stages of the study.

STATISTICAL ANALYSIS

The analysis of peptide YY level, ghrelin level, VAS for hunger and satiety between obese and non-obese groups were presented in graphs and area under the curve (AUC). AUC was calculated using the AutoCAD software program (San Rafael, CA, USA). The non paired t test was used to analyze between obese and non-obese groups for normally distributed numerical data of peptide YY, ghrelin, VAS for hunger and satiety. Meanwhile, Mann-Whitney test was used for abnormally distributed data. Statistical analyses were performed using Statistical Package for the Social Science (SPSS) software version 19.0 for Windows (Chicago, IL, USA) and P-value < 0.5 was considered to be statistically significant.

RESULTS

There were 18 people in the obese group and 22 people in the non-obese group which followed this study. There were no significant differences ($p > 0.05$) in terms of age, educational level, and physical activity between both groups. As expected, BMI and nutritional status had a significant difference ($p < 0.05$). These results were described in Table 1.

First, we examined the Peptide YY and ghrelin in obese and non-obese groups. Both peptide YY level in obese and non-obese group were increased, started at 15 minutes, steadily in the 60 and 120 minutes, and finally reached its peak at 180 minutes after breakfast. The levels of peptide YY during fasting and post-prandial in 15, 60, 120, 180 minutes in the obese group were lower than in the non-obese group (Figure 2). In 15 and 60 minutes after breakfast, the levels of ghrelin in both obese and non-obese groups were declined, compared to the fasting period. Then, it was increased in 120 and 180 minutes after breakfast. Levels of ghrelin during fasting and 15, 120, and 180 minutes after breakfast in obese group were lower than in non-obese group (Figure 3).

Next, we analyzed the degree of hunger and satiety by VAS measurements. VAS for hunger in both groups showed the decreased hunger in 15 minutes after breakfast followed by increased hunger in 60 and 120 minutes and reached the peak in 180 minutes after breakfast as seen in Figure 4. VAS for hunger in obese group during fasting and in 15 minutes after breakfast were lower than in non-obese group. However, in 60, 120, and 180 minutes after breakfast, VAS for hunger in obese group was higher than in non-obese group. As described in Figure 5, VAS for satiety in obese and non-obese group were increased and reached the peak in 15 minutes, but they were declined in 60 to 180 minutes. VAS for satiety during fasting period in obese group was higher than in non-obese group while VAS during post-prandial in 15, 60, and 180 minutes was lower in the obese group, compared to the non-obese group.

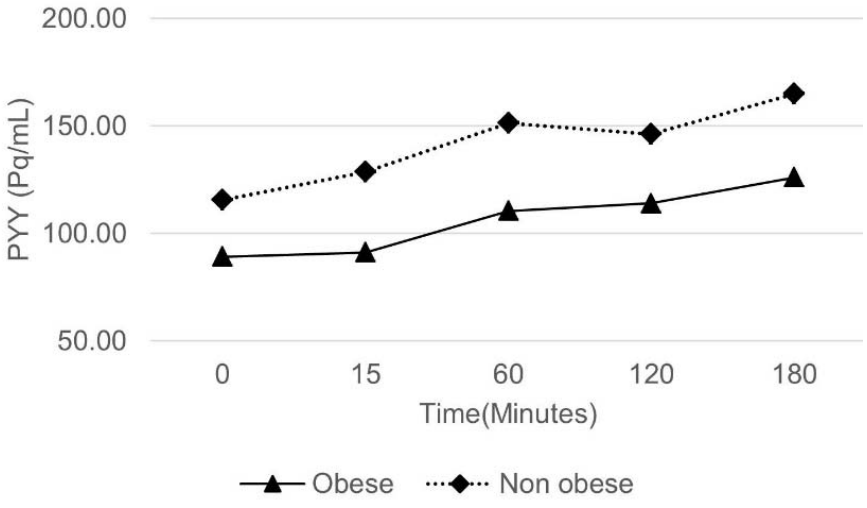

Figure 2. The serial level of Peptide YY in obese and non-obese groups during fasting period and post-prandial in 15, 60, 120 and 180 minutes in obese and non-obese group.

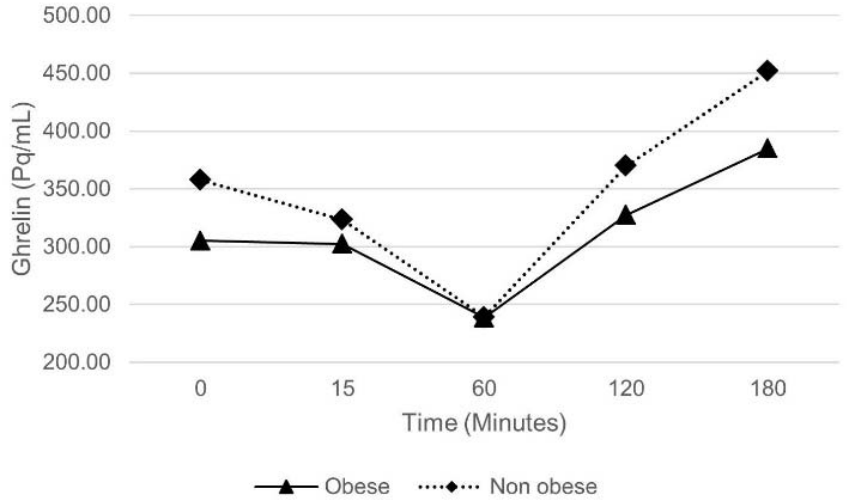

Figure 3. The serial level of Ghrelin in obese and non-obese groups during fasting period, and 15, 60, 120 and 180 minutes post-prandial in obese and non-obese groups.

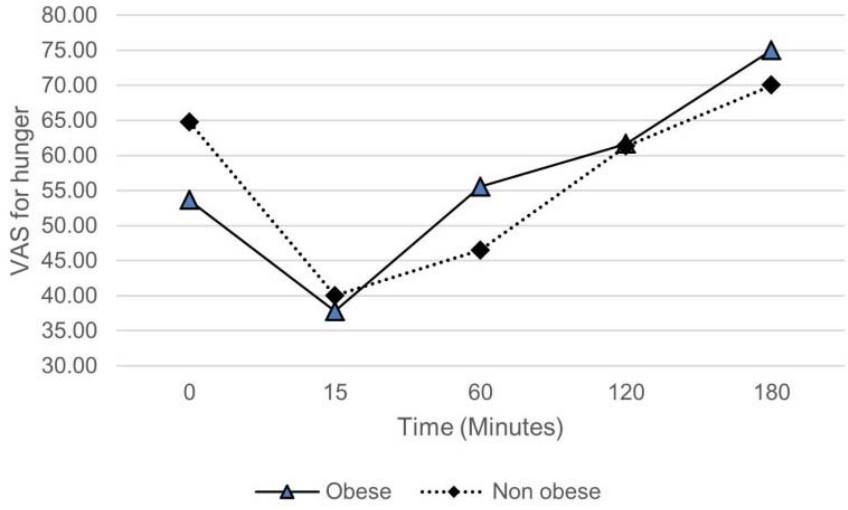

Figure 4. The serial level of VAS for hunger in obese and non-obese groups during fasting period, 15, 60, 120 and 180 minutes post-prandial in obese and non-obese groups.

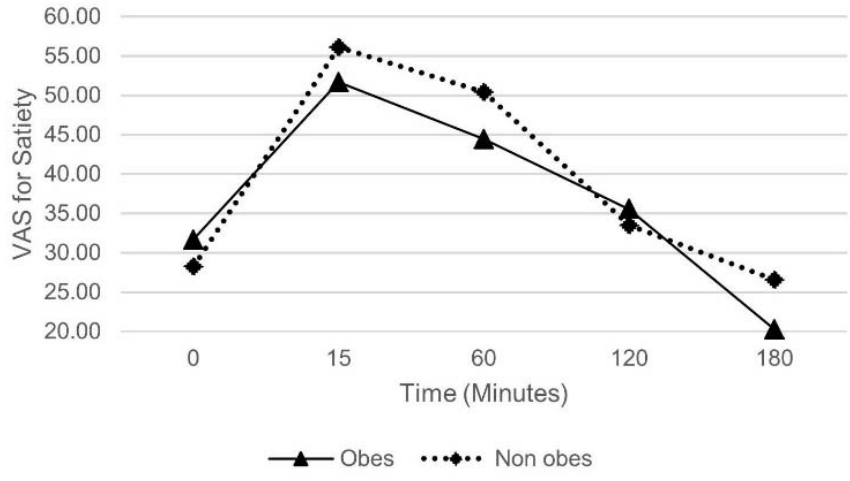

Figure 5. The serial level of VAS for satiety in obese and non-obese groups during fasting period, 15, 60, 120 and 180 minutes post-prandial in obese and non-obese groups.

Table 1
Characteristics of subjects based on age, educational level, physical activity, body mass index (BMI) between the obese and non-obese group

Characteristics	Obese (n = 18)	Non-Obese (n = 22)	*p* value
Age (years), mean (SD)	30.78 ± 5.16	29.27 ± 5.67	0.386[TT]
Educational level, (n,%)			1.000[C]
Low-intermediate	0 (0)	0 (0)	
High	18 (100)	22 (100)	
Physical activity, (n,%)			0.418[C]
Never	10(55.6)	15(68.2)	
Ever	8(44.4)	7(31.8)	
-	-	-	-
BMI, mean (SD)	28.15 ± 1.98	21.11 ± 1.19	< 0.001[TT]
Nutritional status, (n,%)			0.012[KS]
Underweight (BMI ≤ 18.5)	3 (16.7)	10 (45.5)	
Normal (BMI 18.5-22.9)	11 (61.1)	12 (54.5)	
Overweight (BMI ≥ 23)	4 (22.2)	0 (0)	

Notes: [C] Chi-Square Test, [KS]: Kolmogorov-Smirnov MW:Mann-Whitney, TT:Non-pair T test.

Table 2
AUC of PYY, ghrelin, VAS for hunger and satiety in obese and non-obese groups

AUC	Obese	Non-obese	*p* value
PYY	20511.57 ± 7357.94	26723.05 ± 7698.78	0.013*[TT]
Ghrelin	44743.99(19312.80–146377.50)	61945.31 ± 33513.24	0.568[MW]
VAS hunger	10575.00(2100.00–13950.00)	9375.41 ± 2404.74	0.174[MW]
VAS satiety	7137.50 ± 2596.07	7598.86 ± 2027.76	0.542[TT]

Note: *: significant, [MW]: *Mann- Whitney*, [TT]: non-paired T-test. The area under the curves (AUC) were generated by using the AutoCAD software. The AUC of peptide YY in obese group was significantly lower than in the non-obese group ($p < 0.05$)

Table 3
Ad libitum intake 4 hours after balanced composition breakfast of obese and non-obese groups

Variable (kcal)	Obese	% Energy total	Non-obese	% Energy total	*p* value
Energy total	530.60 (423.40–757.30)		421.24±113.06		0.001*[MW]
Protein	99.60 (70.00–164.40)	18.77	72.47±25.39	17.20	0.001*[MW]
Fat	251.10 (166.50)–404.10	47.32	189.20±62.64	44.91	0.001*[TT]
Carbohydrate	182.80 (119.60–219.20)	34.45	159.78±39.20	37.93	0.211[MW]

Note: *: significant, [MW]: *Mann- Whitney*, [TT]: non-paired T-test

We then determined the area under the curve (AUC) of Peptide YY, Ghrelin, and VAS for hunger and satiety. The AUC of peptide YY in the obese group was significantly lower than in the non-obese group ($p < 0.05$). There were no significant differences in AUC of ghrelin, VAS for hunger and satiety in both obese and non-obese group (Table 2).

Finally, we investigated the a*d libitum* intake in obese and non-obese groups during the lunch period (4 hours after breakfast). We found that total energy, protein, and fat intake in obese group were significantly higher than in non-obese group ($p = 0.01$), meanwhile, there was no significant difference in carbohydrate intake ($p = 0.211$), as detailed in Table 3.

DISCUSSION

Altered gut hormones in the obese individuals remain to be elucidated. This study showed that the level of anorexigenic gut hormone peptide YY during fasting and post-prandial period in the obese group was lower than in the non-obese group. AUC was also significantly different between both groups. Low peptide YY AUC reflects the high willingness

for a meal during fasting and during 15, 60, 120, 180 minutes post-prandial among obese subjects. These findings suggest that obesity is not only associated with leptin resistance condition [17]. Low level of peptide YY in the obese group resulted in high VAS for hunger and low VAS for satiety. In one study, when the normal and obese subjects are given a physiological intravenous dose of peptide YY, their intake would be declined more than 30% within 2 hours after the infusion [17].

Although ghrelin is an orexigenic gut hormone, however the level of ghrelin in obese subjects was low in this study and in some previous studies [18, 19]. These results suggest an inverse relationship between BMI, composition, and distribution of visceral fat and fasting or post-prandial ghrelin level. This finding also highlighted the adaptation mechanism in which obese subjects will not gain weight and normal subjects will not lose weight. The regulation disrupted the cholinergic vagus nerve which against decreasing post-prandial ghrelin levels was also found in obese subjects [20].

Insulin resistance which causes hyperinsulinemia generally occurred in obese subjects. Insulin is known to inhibit the signal which secretes ghrelin [21]. Obese subjects have a reduced ghrelin secretion before and after meals. In one trial of low-dose infusion of ghrelin, it was shown that obese subjects have an increase in *ad libitum* energy intake, whereas normal subjects have not an increasing intake [22]. The study suggests that obese subjects have high sensitivity against the ghrelin effect in increasing hunger, in spite of the low levels of ghrelin circulation. In thin subjects, however, they have higher levels of ghrelin during fasting period and prominent declining levels of ghrelin after meals. Thin subjects have a greater hunger before meals, but have a faster satiety after meals.

Hunger and satiety were measured by VAS. In obese subjects, VAS for hunger during fasting period was lower and VAS for hunger during post-prandial period was higher than in non-obese subjects. VAS for satiety during fasting period in obese subjects was higher and VAS for satiety during post-prandial period was lower than in non-obese subjects. The results of hunger and satiety with VAS measurements were consistent in this study. This was also shown in AUC, VAS for hunger and satiety in obese subjects has tended to be lower than in non-obese subjects, although it did not reach the statistically significant threshold. Taken together, these findings confirmed the power of repetition, the strength of the research, and the validity of the VAS to assess hunger and satiety [14, 15].

The *ad libitum* lunch intake analysis revealed that obese subjects have higher intake than non-obese, especially in fat intake, but not in carbo-hydrate. Satiety was assessed calculating the *ad libitum* food calorie intake after 4 hours from breakfast. That method was the high reproducibility in food intake assessment and was not affected by food intake one day earlier [23]. The high intake in obese subjects was consistent with the level of VAS for hunger and satiety.

Based on the results of gut hormone profile, VAS for hunger, VAS for satiety, and *ad libitum* intake, we suggest that anorexigenic gut hormone has a tendency of inverse relationship with VAS for satiety. *Ad libitum* intake analysis in obese subjects was appropriate with higher VAS for hunger and lower VAS for satiety. It was also appropriate with the level of peptide YY, but not in the level of ghrelin.

CONCLUSION

The current study highlighted the roles of peptide YY and ghrelin in the regulation of hunger and satiety in obese women. To the best of our knowledge, our study is the first to reveal lower level of peptide YY, ghrelin and satiety, and a higher level of hunger and *ad libitum* intake 4 hours after breakfast in obese Indonesian women, as compared to non-obese women. In the future treatment of obesity, peptide YY may be considered as a potential treatment to suppress hunger.

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Conflict of interest disclosure: The authors declare that there are not conflicts of interest.

Author contributions: **Witjaksono F**: Conceptualization, Data Curation, Funding Acquisition, Formal Analysis, Investigation, Methodology, Project Administration, Resources, Manuscript Writing and Final Approval of the Manuscript; **Simadibrata M**:

Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Manuscript Editing, and Final Approval of the Manuscript; **Wijaya L**: Formal Analysis, Methodology, Manuscript Editing and Final Approval of the Manuscript; **Wijaya A**: Funding Acquisition, Investigation, Project Administration, Resources, and Final Approval of the Manuscript; **Nurwidya F**: Formal Analysis, Methodology, Manuscript Editing, and Final Approval of the Manuscript.

Introducere. *Studiul de față şi-a propus să evalueze profilul peptidului YY şi al grelinei precum şi scara analogă vizuală (VAS) pentru senzația de foame şi saţietate precum şi aportul ad libitum comparativ la femeile obeze şi non-obeze.*

Metode. *A fost realizat un studiu clinic intervențional nerandomizat ce a inclus femei obeze (BMI ≥ 25–35 kg/m^2) şi femei fără obezitate (BMI 18.5–23.0 kg/m^2) . Nivelurile peptidului YY şi ale grelinei au fost măsurate folosind tehnica ELISA iar senzația de foame şi saţietate au fost evaluate printr-o VAS. Rezultatele au fost comparate în condiții de post precum şi în dinamică la 15, 60, 120 şi 180 de minute după un mic dejun standardizat. Studiul a evaluat şi aportul ad libitum la 4 ore după micul dejun.*

Rezultate. *Comparativ cu grupul non-obez pacientele obeze au avut niveluri semnificativ mai mici ale peptidului YY atât în momentul postului cât şi la 16, 60, 120 şi 180 de minute după micul dejun, cu niveluri mai mici ale AUC pentru acest biomarker. În plus, grupul de paciente obeze au avut aport ad libitum semnificativ statistic mai mare. Pacientele obeze au avut niveluri serice mai mici ale grelinei şi scoruri VAS mai mici pentru senzația de foame şi mai mari pentru senzația de saţietate comparativ cu pacientele non-obeze.*

Concluzii. *Au fost relevate diferențe semnificative între nivelurile peptidului YY, ale grelinei, ale scalelor VAS pentru saţietate şi senzația de foame precum şi pentru aportul ad libitum la 4 ore după micul dejun.*

Correspondence to: Fiastuti Witjaksono, MD., M.Sc., Ph.D. Department of Nutrition, Faculty of Medicine Universitas Indonesia Cipto Mangunkusumo Hospital, Jakarta, Indonesia. Jl. Salemba Raya No. 6, Jakarta 10430, Indonesia, Telephone: +62-813-1082-0451. Fax: +62-21-315-2532
E-mail: fiastuti_dr@yahoo.com

REFERENCES

1. INDONESIAN MINISTRY OF HEALTH [Kementerian Kesehatan Republik Indonesia]. Basic Health Research [Riset Kesehatan Dasar, Riskesdas 2013]. Jakarta; 2013. Available in: http://www.depkes.go.id/resources/download/general/Hasil%20Riskesdas%202013.pdf. Last accessed: 5 May 2018.
2. SONESTEDT E., ROOS C., GULLBERG B., ERICSON U., WIRFALT E., ORHO-MELANDER M. *Fat and carbohydrate intake modify the association between genetic variation in the FTO genotype and obesity*. Am J Clin Nutr. 2009;**90**(5):1418-25.
3. ELFHAG K., ROSSNER S. Weight loss maintenance. In: Kopelman PG, Caterson ID, Dietz WH, editors. *Clinical obesity in Adult and Children*. 2 ed. Massachusetts: Blackwell Publishing; 2005:407-20.
4. MURPHY KG., BLOOM SR. *Gut hormones in the control of appetite*. Exp Physiol. 2004;**89**(5):507-16.
5. RAYBOULD HE., MEYER JH., TABRIZI Y., RODGER A., TSO L., TSO P. *Inhibition of gastric emptying in response to intestinal lipid is dependent on chylomicron formation*. Am J Physiol Regulatory Integrative Comp Physiol. 1998;**274**:1834-8.
6. DULLOO AG. Energy balance and body weight homeostasis. In: Kopelman PG, Caterson ID, Dietz WH, editors. *Clinical Obesity in Adult and Children*. 2 ed. Massachusetts Blackwell Publishing; 2005, p. 67-80.
7. STANLEY S., WYNNE K., MCGOWAN B., BLOOM S. *Hormonal regulation of food intake*. Physiol Rev. 2005;85:1131-58.
8. ESSAH PA., LEVY JR., SISTRUM SN., KELLY SM., NESTLER JE. *Effect of Macronutrient Composition on Postprandial Peptide YY Levels*. J Clin Endocrinol Metab 2007;**92**(10):4052-55.
9. SHERWOOD L. Energy balance and temperature regulation. *Human Physiology: From Cells to Systems*. 7 ed: Thomson, Belmont; 2010, p. 641-59.
10. BLUNDELL JE., FINLAYSON G., HALFORD J. Biology of Obesity: Eating behaviour. In: Kopelman PG, Caterson ID, Dietz WH, editors. *Clinical Obesity in Adult and Children*. 2 ed. Massachusetts: Blackwell Publishing; 2005, p. 137-48.
11. TRITOS NA., KOKKOTOU EG. *The physiology and potential clinical applications of ghrelin, a novel peptide hormone*. Mayo Clin Proc. 2006;**81**(5):653-60.

12. CUMMINGS DE., WEIGLE DS., FRAYO RS., BREEN PA., MA MK., DELLINGER EP., *et al*. *Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery*. N Engl J Med. 2002;**346**(21):1623-30.
13. ROGERS PJ., BLUNDELL JE. *Effect of anorexic drugs on food intake and the micro-structure of eating in human subjects.* Psychopharmacology (Berl). 1979;**66**(2):159-65.
14. RABEN A., TAGLIABUE A., ASTRUP A. *The reproducibility of subjective appetite scores*. Br J Nutr. 1995;**73**(4):517-30.
15. FLINT A., RABEN A., BLUNDELL JE., ASTRUP A. *Reproducibility, power and validity of visual analog scales in assessment of appetite sensations in single test meal studies*. Int J Obes Relat Metab Disord. 2000;**24**(1):38-48.
16. STUBBS RJ., HUGHES DA., JOHNSTONE AM., ROWLEY E., REID C., ELIA M., *et al. The use of visual analog scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings*. Br J Nutr. 2000;**84**:405-15.
17. BATTERHAM RL., COHEN MA., ELLIS SM., LE ROUX CW., WITHERS DJ., FROST GS., *et al. Inhibition on food intake in obese subject by peptide YY 3-36*. N Engl J Med. 2003;**349**:941-8.
18. BOWEN J., NOAKES M., CLIFTON PM. *Appetite regulatory hormone responses to various dietary proteins differ by body mass index status despite similar reductions in ad libitum energy intake*. J Clin Endocrinol Metab. 2006;**91**:2913-9.
19. TENTOLOURIS N., KOKKINOS A., TSIGOS C. *Differential effects of high-fat and high-carbohydrate content isoenergetic meals on plasma active ghrelin concentrations in lean and obese women*. Hormone Metabol Res. 2004;**36**:559-63.
20. MAIER C., RIEDL M., VILA G. *Cholinergic regulation of ghrelin and peptide YY release may be impaired in obesity*. Diabetes. 2008;**57**:2332-40.
21. CUMMINGS DE., PURNELL JQ., FRAYO RS., SCHMIDOVA K., WISSE B., WEIGLE DS. *A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans*. Diabetes. 2001;**50**:1714-19.
22. DRUCE MR., WREN AM., PARK AJ. *Ghrelin increases food intake in obese as well as lean subjects*. Inter J Obes. 2005;**29**:1130-36.
23. GREGERSEN NT., FLINT A., BITZ C., BLUNDELL JE., RABEN A., ASTRUP A. *Reproducibility and power of ad libitum energy intake assessed by repeated single meals*. Am J Clin Nutr. 2008;**87**:1277-81.

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