

2019

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Recommended Citation

Handayani, Dian; Kusumastuty, Inggita; Andarini, Sri; Sabrina, Nindy; Rudijanto, Achmad; and Huang, Xu-Feng, "Sensitivity of plasma cholecystokinin and peptide YY in obese and normal weight men" (2019). *Illawarra Health and Medical Research Institute*. 1490.
<https://ro.uow.edu.au/ihmri/1490>

Sensitivity of plasma cholecystokinin and peptide YY in obese and normal weight men

Abstract

Introduction: Cholecystokinin (CCK) and peptide YY (PYY) are satiety-stimulating hormones that are released during eating. As such, their levels may be used useful in obesity intervention. The aims of this study were to determine the optimal cutoff values, sensitivity and specificity of plasma CCK and PYY in adult men, in order to determine hormonal dysfunction in obesity. **Methods:** We investigated 16 obese [body mass index (BMI) ≥ 25.1] and 16 normal weight (BMI 18.5–22.9) men. They ate isocaloric fast-food for breakfast. Blood for the determination of the hormones was collected at 0 (before), 30, 60, and 120 minutes after consumption. The data that was obtained were analysed using an independent t-test or the Mann–Whitney U-test. The receiver operating characteristic (ROC) curve was drawn and the trapezoidal rule analysis was performed to determine the area under the curve, to determine the optimal cut-off values, sensitivity and specificity. **Results:** In obese subjects, CCK was lower compared with normal weight subjects at any time ($p < 0.05$). There were no major differences in PYY among subject groups. ROC curve analysis demonstrated that the plasma CCK had an optimal cut-off of 6,310 pg/ml at 120 minutes after eating, with 0.97 area under curve (AUC), sensitivity was 94%, and specificity was 94%. The cut-off for optimal PYY was an average of 294.5 pg/ml at 120 minutes after eating (AUC 0.74; sensitivity 75%; specificity 75%). **Conclusion:** Our findings suggest that the plasma CCK level is a better potential predictor of obesity and constantly decreased over time compared to PYY.

Disciplines

Medicine and Health Sciences

Publication Details

Handayani, D., Kusumastuty, I., Andarini, S., Sabrina, N., Rudijanto, A. & Huang, X. (2019). Sensitivity of plasma cholecystokinin and peptide YY in obese and normal weight men. *Malaysian Journal of Nutrition*, 25 (2), 217-226.

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Sensitivity of plasma cholecystokinin and peptide YY in obese and normal weight men

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ABSTRACT

Introduction: Cholecystokinin (CCK) and peptide YY (PYY) are satiety-stimulating hormones that are released during eating. As such, their levels may be used useful in obesity intervention. The aims of this study were to determine the optimal cut-off values, sensitivity and specificity of plasma CCK and PYY in adult men, in order to determine hormonal dysfunction in obesity. **Methods:** We investigated 16 obese [body mass index (BMI) ≥ 25.1] and 16 normal weight (BMI 18.5–22.9) men. They ate isocaloric fast-food for breakfast. Blood for the determination of the hormones was collected at 0 (before), 30, 60, and 120 minutes after consumption. The data that was obtained were analysed using an independent *t*-test or the Mann-Whitney U-test. The receiver operating characteristic (ROC) curve was drawn and the trapezoidal rule analysis was performed to determine the area under the curve, to determine the optimal cut-off values, sensitivity and specificity. **Results:** In obese subjects, CCK was lower compared with normal weight subjects at any time ($p < 0.05$). There were no major differences in PYY among subject groups. ROC curve analysis demonstrated that the plasma CCK had an optimal cut-off of 6,310 pg/ml at 120 minutes after eating, with 0.97 area under curve (AUC), sensitivity was 94%, and specificity was 94%. The cut-off for optimal PYY was an average of 294.5 pg/ml at 120 minutes after eating (AUC 0.74; sensitivity 75%; specificity 75%). **Conclusion:** Our findings suggest that the plasma CCK level is a better potential predictor of obesity and constantly decreased over time compared to PYY.

Keywords: Cholecystokinin, peptide yy, obese, receiver operating characteristic

INTRODUCTION

The high prevalence of obesity has emerged as a health concern since it is a risk factor of chronic disease (Heymsfield & Wadden, 2017). In Indonesia, 19.7%

of the men and 32.9% of the women were obese in 2013 (NIHRD, 2013). The combined prevalence of men and women increased to 21.8% in 2018 (NIHRD, 2018). Between 2013 and 2018, overall central obesity increased by about 5.0%

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doi: <https://doi.org/10.31246/mjn-2018-0112>

(26.6% to 31.0%). Excessive energy intake, as the cause of obesity, involves the brain-gut axis in the central nervous system. Hormones such as peptide YY (PYY) and cholecystokinin (CCK) play a role in decreasing appetite (Posovszky & Wabitsch, 2015).

PYY and CCK have been known as satiety hormones contributing to the delay and inhibition of gastric emptying (Adamska *et al.*, 2014). In humans, low levels of PYY have been associated with obese patients (Batterham *et al.*, 2003). An animal study found low PYY levels in obese rats and high PYY in lean rats, suggesting that PYY deficiency plays a role in the pathogenesis of obesity (Moghadam, Moran & Dailey, 2017). A human study also showed that CCK concentrations in metabolic syndrome that followed morbid obesity were significantly lower than those in lean control subjects (Zwirska-Korczala *et al.*, 2007). It can thus be inferred that the alteration in PYY and CCK hormones are intimately associated with obesity.

The gut hormones have been investigated as a potential therapeutic target for obesity. Recently, acylated ghrelin (AG), an energy homeostasis regulator hormone, has been one of the targets for obesity therapy using ghrelin O-acyltransferase (GOAT) inhibitor (Khatib *et al.*, 2015). Andarini, Kangsaputra & Handayani (2017) investigated the AG levels and optimal cut-off values in normal and obese patients using receiver-operating characteristic (ROC) curve analysis. The investigation revealed that there was a significant difference in AG levels, and the optimal cut-offs were 2,332 pg/ml before eating (sensitivity 88%; specificity 100%) and 2,710 pg/ml at 30 minutes after eating (sensitivity 88%; specificity 100%) (Andarini *et al.*, 2017). Determination of gut hormone cut-offs would assist in establishing rational therapeutic principles in obesity therapy

management. Some researchers have found that appetite control hormones, CCK and PYY, can also be potential therapeutic targets in the management of obesity (Perry & Wang, 2012; Olszanecka-Glinianowicz *et al.*, 2013; Prinz & Stengel, 2017). CCK and PYY deficiency in obese subjects, treatment with CCK and PYY has been attempted. However, an animal study demonstrated that chronic CCK administration induced pancreatitis in rats, and high dose PYY infusion produces vomiting and nausea (Degen *et al.*, 2005; Jia, Yamamoto & Otsuki, 2015).

The current literature provides limited information on CCK and PYY cut-off levels. This aim of this study was to investigate CCK and PYY levels between obese men and control, and to assess the optimal cut-off, sensitivity and specificity of CCK and PYY by using ROC analysis.

MATERIALS AND METHODS

Study design

This study was conducted in Malang, Indonesia. It aimed to obtain and compare the optimal cut-off values, sensitivity, and specificity of CCK and PYY plasma levels using ROC analysis to evaluate pre- and post-prandial plasma CCK and PYY. Participants were given isocaloric food (51% carbohydrate, 33% fat, and 13% protein) and water for breakfast after overnight fasting. Fasting blood samples were collected before breakfast (0 minutes), and 30, 60, and 120 minutes after breakfast. The study was approved by the Ethical Committee of Medical Faculty of Universitas Brawijaya (No. 389/EC/KEPK/2015).

Test meals

One serving of commercial fast-food containing similar amounts of energy (546–593 kcal) was provided to the 32 participants at breakfast time. The

meals were obtained from a popular fast-food franchise restaurant in Malang, Indonesia. This study used commercial fast-food since the community expressed a preference for it.

Previous studies have reported that the consumption of fast-foods correlated with obesity (Janet *et al.*, 2010; Andreyeva, Kelly & Harris, 2011). The total energy and macronutrient content are presented in our previous study (Handayani *et al.*, 2017). The amount of energy was analysed using a bomb calorimetric, protein was assayed by the Kjeldahl method and fibre by enzymatic analysis.

Participants

Sixteen men with normal weight and 16 obese men were recruited. Males were chosen as the participants due to their stable energy intake, since females have a fluctuating energy intake within their menstrual cycle. The physiological responses to hunger and satiety also differ between male and female (Bédard *et al.*, 2015). Pre-screening was performed to determine healthy participants without a previous history of diabetes mellitus, hypercholesterolemia, or hypertension. Blood pressure, blood glucose and plasma cholesterol levels were measured using sphygmomanometer, blood glucose test kit and the multi-monitoring system Autocheck FDA-CE197, respectively. Plasma leptin was measured using competitive enzyme-linked immunosorbent assay (ELISA) (KIT: CAN-L-4260, DB Canada). The percentage (%) body weight and % waist fat were measured using bioelectrical impedance analysis (BIA)-Omron type HBF 375. All participants provided informed consent for the study.

Blood biochemistry examination

Fasting blood samples were collected for CCK and PYY from participants before eating, and at 30, 60 and 120

minutes after. Plasma was separated from blood samples by centrifugation at 3,000 revolutions per minute (rpm) for 10 minutes at 25°C using PLC-05 tabletop centrifuge (Gammy Industrial Corporation, Taiwan, in association with Cannic, Inc., USA) and then was stored at -80°C until analysis.

Plasma CCK (pg/ml) was measured using competitive ELISA (E-EL-H0723; Elabscience, Biotechnology, Beijing). Plasma PYY (pg/ml) was measured using sandwich ELISA (E-EL-H1237; Elabscience, Biotechnology, Beijing). The plasma samples were added onto a pre-coated microtiter plate and combined with biotinylated detection antibody. After incubation at 37°C, horseradish peroxidase (HRP) conjugate was added and incubated. The substrate reagent was added for the colour reaction. The optical density (OD) was measured at a wavelength of 450.0±2.0nm. CCK and PYY hormone levels were obtained from the OD of the samples and the standard curve.

Statistical analysis

The data are presented as a mean±standard deviation (SD). The baseline comparison between obese and normal weight men was examined using the independent *t*-test. For CCK and PYY levels, differences between obese and normal weight were examined by using the independent *t*-test for normally distributed data and Mann-Whitney U test for abnormally distributed data. The area under the curve (AUC) of CCK and PYY was performed by calculating the graphic trapezoid. The AUC scores were interpreted as excellent if ≥0.90, good if 0.80–0.89, fair if 0.70–0.79, and poor if <0.70 (Kendzor, Caughy & Owen, 2012). The optimal cut-off values of CCK and PYY were determined using the ROC curve. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version

Table 1. Characteristics of research participants

Characteristics	Obese (n=16) (Mean±SD)	Normal weight (n=16) (Mean±SD)	p-value
Age (years)	21.4±1.9	20.6±1.1	0.18
Weight (kg)	97.0±1.9	60.9±5.7	<0.01
Height (cm)	170.0±5.5	169.0±6.3	0.89
BMI (kg/m ²)	33.6±4.8	21.2±1.1	<0.01
Body fat (%)	30.2±3.8	17.0±5.1	<0.01
Waist fat (%)	16.7±4.1	4.7±1.0	<0.01
Waist circumference (cm)	105.0±12.0	75.4±4.2	<0.01
Systolic blood pressure (mm/Hg)	124.0±9.6	117.0±6.8	0.04
Diastolic blood pressure (mm/Hg)	80.6±4.4	75.0±5.2	<0.01
Random blood sugar (g/dL)	122.0±21.1	103.0±12.2	0.16
Total cholesterol (g/dL)	135.0±27.7	94.8±49.8	<0.01
Leptin (pg/mL)	61.9±21.8	21.6±9.1	<0.01

16 (IBM, Chicago, IL, USA). Statistical analyses were considered as significant at $p<0.05$.

RESULTS

Characteristics of the research participants

In total, 32 adult men (16 normal weight and 16 obese) were recruited for the study. The mean age was 21.4±1.9 years for the obese men and 20.6±1.1 years for the normal weight men. Obese men were heavier and had significantly higher ($p<0.01$) BMI (≥ 25.1), body fat percentage, and waist fat percentage than did normal weight men whose BMI was 18.5–22.9 (Table 1). The obese

men had higher levels of systolic blood pressure, diastolic blood pressure (DBP), total cholesterol, and leptin (Table 1) (Andarini *et al.*, 2017).

CCK and PYY

The mean±SD of CCK and PYY levels in time points are shown in Table 2. In CCK, there was significant difference in the mean values of the obese and normal weight groups ($p<0.002$). Moreover, there was a decrease in CCK concentration in the obese group at the different time points (before eating, 30 minutes after eating, 60 minutes after eating, and 120 minutes after eating) but this did not show in the normal weight group. There

Table 2. CCK and PYY concentrations before eating and 30, 60, and 120 minutes after eating

Variables	Obese (Mean±SD)	Normal weight (Mean±SD)	p-value
CCK 0 min (pg/ml)	7045.6±738.1	8234.4±950.8	<0.002
CCK 30 min (pg/ml)	6371.8±738.1	8033.1±1334.3	<0.001
CCK 60 min (pg/ml)	6195.0±746.7	8395.6±1134.1	<0.001
CCK 120 (pg/ml)	5275.0±789.6	8799.4±1595.9	<0.001
PYY 0 min (pg/ml)	251.9±190.8	155.9±92.3	0.036
PYY 30 min (pg/ml)	279.0±190.8	346.9±113.9	0.121
PYY 60 min (pg/ml)	478.8±263.0	363.1±137.2	0.129
PYY 120 min (pg/ml)	248.3±187.6	353.1±119.1	0.069

Table 3. Cut-off, sensitivity, and specificity of CCK and PYY content before and after eating at each time point

Time (min)	CCK				PYY			
	Cut off (pg/ml)	AUC	Sensitivity	Specificity	Cut off (pg/ml)	AUC	Sensitivity	Specificity
0	7665	0.82	75%	75%	191.5	0.27	38%	38%
30	7040	0.82	75%	75%	266.5	0.31	69%	69%
60	7015	0.96	88%	88%	369.0	0.37	44%	44%
120	6310	0.97	94%	94%	294.5	0.74	75%	75%

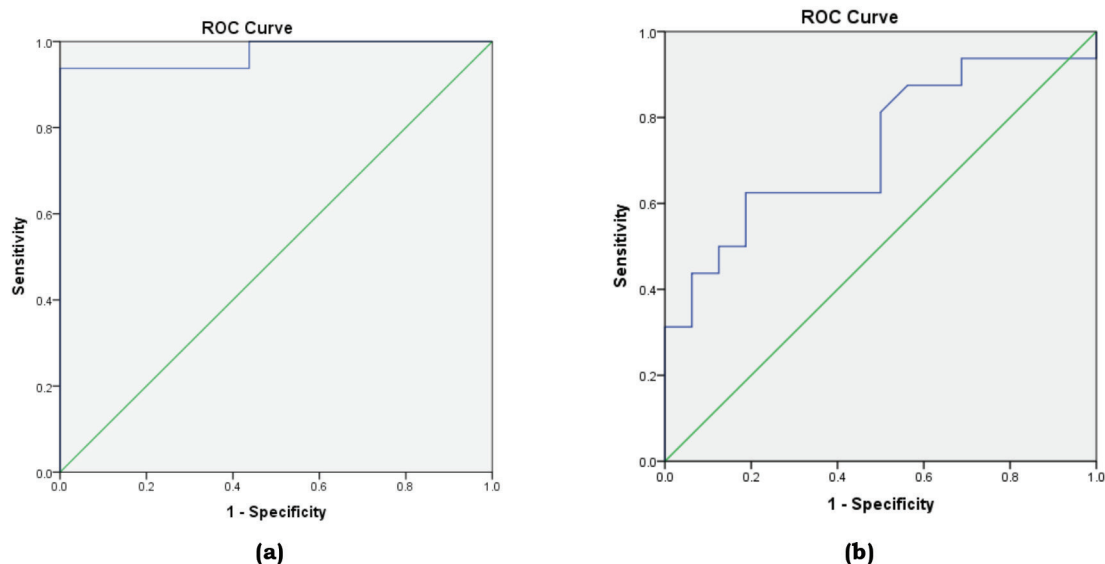


Figure 1. Receiver characteristics curve (ROC) of CCK (a) and PYY (b) at 120 minutes after eating

were no significant differences in PYY levels between the obese and normal weight groups.

CCK and PYY as an alternative biomarker test for obesity

Table 3, Figure 1(a), and Figure 1(b) shows optimal cut-off points and area under ROC curve for CCK and PYY on each time point. The ROC curve of CCK and PYY showed the highest sensitivity and specificity at 120 minutes after eating. ROC analysis of CCK showed a cut-off value of 6,310 pg/ml, with area under curve (AUC) of 0.97, sensitivity was 94% and specificity was 94%. The highest PYY levels showed highest

sensitivity and specificity with a cut-off value of 294.5 pg/ml (AUC, 0.74; sensitivity, 75%; specificity, 75%).

DISCUSSION

In recent years, the alteration of gut hormones that are related to obesity such as CCK and PYY has been documented. Those hormones play an important role in mediating satiation. The present study revealed that when sensitivity and specificity were 94% each, the optimal cut-off value of CCK was 6,310 pg/ml at 120 minutes after eating. At the same time, the optimal cut-off of PYY was at a lower sensitivity and specificity value

(75% and 75%, respectively) than CCK. There was a statistically significant difference between the CCK levels in obese and normal weight men at each of the time points of 0, 30, 60, and 120 minutes, suggesting that obese people have lower CCK levels. However, there were no significant differences in PYY. The increasing CCK concentration indicates a lower gastric emptying rate through an appetite suppression mechanism and food intake reduction. Gastric motility will be suppressed by CCK release, resulting in slower gastric emptying which leads to prolonged gastric distension and satiety (Ma *et al.*, 2009; Li, Ma & Wang, 2011; Robert *et al.*, 2015). Plasma CCK increased within 15 minutes after meal initiation, went higher in 30 minutes and remained elevated over the 120 minutes initiation, and then decreased gradually. Our study confirmed that the decreased CCK concentration at time points contributes to a decrease of satiety and an increase of hunger in obese subjects (Ma *et al.*, 2009; Adamska *et al.*, 2014; Robert *et al.*, 2015).

Obesity has been significantly associated with rapid gastric emptying rates (Acosta *et al.*, 2015). In this study, CCK levels were reported to be lower in obese than in normal-weight men. Our observations were consistent with those of Lean & Malkova (2016) who reported decreased CCK in obese and overweight compared to lean subjects after dietary intervention. Even though Brennan *et al.* (2012) demonstrated no major differences in CCK responses to macronutrients between lean and obese subjects, high protein content stimulated CCK production in their obese respondents compared to high fat. Another investigation found a delayed CCK response to oleic acid infusion in obese subjects compared to control groups (Stewart *et al.*, 2011). These

observations suggest that CCK in obese subjects is sensitive to protein and fat intake. Our finding confirms that the high-fat content in fast foods contributed to lower CCK in obese subjects compared to lean subjects. Also, some investigations found a synergistic interaction between CCK and leptin that resulted in short-term satiety. Heldsinger *et al.* (2011) established that the Phosphoinositide 3-kinase (PI3K) and Signal transducer and activator of transcription 3 (STAT3) signaling pathway can be mediated for CCK and leptin to activate vagal neurons which may contribute to food intake. The significant difference in leptin between obese and lean subjects may affect CCK in controlling satiation. Plasma leptin level in this study as reported in Table 1 is higher in the obesity group compared to the normal weight group.

The amount of CCK gradually decreased over time but increased in sensitivity and specificity with a peak at 120 min following the consumption of food. The mechanism underlying the increases of sensitivity and specificity over time remains unclear. However, the previous finding which revealed the inverse relationship between hunger and plasma CCK but not PYY explains that CCK is more sensitive in controlling satiety (Brennan *et al.*, 2012). The result was consistent with the present study that PYY does not have good specificity and sensitivity as CCK, and the ROC curve appears to fluctuate. The better specificity and specificity over time in CCK also showed that time could determine the amount CCK released into circulation.

PYY acts in the reduction of gastric emptying and the delay in intestinal transit, which is called 'ileal brake'. PYY also has biological functions including reducing food intake, decreasing gastric emptying, and slowing gastrointestinal motility. The lack of endogenous PYY

secretion may lead to obesity development (Li *et al.*, 2011; Adamska *et al.*, 2014; Troke, Tan & Bloom, 2014). Some studies reported that obese subjects have lower fasting and postprandial plasma PYY than normal weight subjects. It has been reported that protein or fat has the strongest stimulant of PYY secretion and thus contribute to its increase in blood concentrations (Mittelman *et al.*, 2010; Li *et al.*, 2011; Lean & Malkova, 2016). The obese subjects had significantly higher hunger and lower satiety than the normal weight subjects. However, the postprandial PYY profiles were not translated into the feeling of less hungry or more fullness that led to a reduction in food intake (Lomenick, Clasey & Anderson, 2008; Lomenick *et al.*, 2009; van der Klaauw *et al.*, 2013). The PYY response was reported slightly higher immediately after exercising both in obese men and women. Lifestyle and environment factors do not have a significant impact on plasma PYY in men, but some factors such as smoking, medication use, and a post-menopause condition in women lead to an increase of circulating PYY concentration (Cahill *et al.*, 2014; Lean & Malkova, 2016). Our data were also consistent with the latest study in older and middle-aged men by Madsen *et al.* (2019) who found that there was no difference in plasma concentration of PYY in response to lipid load. The 30-minute interval of test meal had been chosen based on previous studies which showed a significant difference in gut hormones (le Roux *et al.*, 2006).

This study had several limitations. The first limitation was the small number of participants in each group. Secondly, even though the study used fast food meals with similar energy density, the serving size among the foods was different. A previous study revealed that food form and portion size

affect postprandial hormonal response in normal weight and obese subjects (Leidy *et al.*, 2010). It is suggested that in future studies, the confounding factor of the test meal could be minimised by choosing the same food with similar serving size and macronutrient content. In this way, the focus would be more on analysing the sensitivity and specificity of the hormonal response.

This study showed that CCK is more sensitive and specific than PYY in the study of gut hormones in obese subjects. This finding may enhance the development of appropriate gut hormonal testing and intervention to help to combat obesity.

CONCLUSION

At present, CCK is rarely considered as a gut hormone target in obesity management. However, we have established that CCK is better than PYY in predicting obesity from the significantly different amounts of CCK in obese and lean subjects at all time points. Furthermore, we have demonstrated that CCK has better sensitivity and specificity over time, unlike PYY, which had a fluctuating ROC curve. Our findings suggest that CCK has potential in obesity treatment. Future studies are required to clarify the nutrients and hormones contributing to CCK release and the side effect(s) of CCK administration.

Acknowledgement

This research was funded by Universitas Brawijaya. The Ministry of Research, Technology, and Higher Education, Republic of Indonesia provided a grant (No. 007/Add/SP2H/PL/DIT. LITABMAS/V/2015) for this study.

Authors' contributions

DH, the principal investigator, conceptualised and designed the study, prepared the draft of the manuscript and reviewed the manuscript; IK, led the data collection and statistical analysis; SA, led

the data collection and statistical analysis; AR and NS, assisted in drafting the manuscript, advised on the data analysis and interpretation and reviewed the manuscript; XFH, conducted data analysis and interpretation, assisted in drafting the manuscript and reviewed the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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