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Altered Baseline of Plasma Glucagon Level in pre-Obese to very Obese Persons

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Abstract

"Bihormonal hypothesis" is disturbances of both insulin and glucagon in diabetes mellitus. It resulted in a high blood glucose level. Interestingly, as one of Diabetes Mellitus's risk factors and metabolic disorders, obesity may also play role in altering homeostasis and regulation of glucose metabolism in blood and its utilization in tissues. Unfortunately, there is limited information about the alteration of glucagon levels in various degrees of obesity. This research objective is to learn the plasma glucagon levels alteration in pre-obese, obese, and very obese persons in Jatinangor in 2015. We had observed 31 obese female subjects in one village. This study was conducted using descriptive quantitative with cross-sectional design. Blood vein samples from the left arm were collected, stored, and transferred to Dr.Hasan Sadikin General Hospital. Glucagon plasma was measured by the ELISA method. We discovered an interesting pattern that showed a correlation between glucose level and the glucagon level in a very obese group. We observed average glucose level is declined and linearly associate with the glucagon level from pre obese to obese and to very obese group. The average level of glucagon in the pre-obese group is 158.62 pg/mL, the obese group is 149.99 pg/mL, and the very obese group is 111.98 pg/mL.

Introduction

The prevalence of obesity has been increasing for the last 50 years. In Indonesia, based on Riskesdas 2013, obesity prevalence increased significantly. Prevalence of obesity in adult females (>18 years) inclined from 15,5% in 2010 to 32,9% in 2013. The obesity prevalence in males also increased from 7.8% in 2010 to 19.7% in 2013. Different from WHO classification, Riskesdas classifies obesity when the BMI reaches 27 kg/m2, while WHO 30 kg/m2 (Riset Kehatan Dasar, 2013). This is due to Asians have a risk of hypertension, diabetes, dyslipidemia with lower BMI than WHO recommends (Wells and Victoria, 2005). Obesity is one of the factors of metabolic syndrome, which is closely related to various diseases, such as cardiovascular disease and diabetes mellitus type 2. There is a close relationship between obesity and DM type 2.

Fifteen thousand six hundred eighty people, ranging from age 35 – 74, were observed for at least eight years. Interestingly, 28.3% of males with obesity and 31.3% female with obesity then have diabetes mellitus type 2 (Wang et al., 2014). It is due to the adipose tissue in people with obesity can secrete pro-inflammation factors, which have a great role in the development of insulin resistance (Nehete et al., 2014). In people with diabetes, there are

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disturbances in both of secretion and function of insulin which causes the elevation of blood glucose and FFA (Golay et al., 1986).

Physiologically, two hormones work antagonistically to regulate the homeostasis of blood glucose. They are insulin and glucagon. Insulin is the hormone secreted by the beta cell of the pancreas. Its function mainly is to maximize glucose transportation from blood to all over the body cells to be utilized then as a source of energy. In the condition the blood glucose is too high, insulin acts in glycogenesis in the liver and skeletal muscle (Hall, 2006). Glucagon is the hormone secreted by the alpha cell of the pancreas. Its function is to increase blood glucose through its actions in the liver. Glucagon increases glycogenolysis and gluconeogenesis in the liver (Quesada et al., 2008). Interestingly, in 1975, Unger and Orci published "bihormonal hypotheses" which stated that there was a combination between insulin and glucagon disturbances in diabetes mellitus (Quesada et al., 2008).

Early hyperglucagonemia correlated with hyperinsulinemia and lipid metabolic disturbances (Manell, 2019). Another research revealed hyperglucagonemia in people with diabetes mellitus type 2 (Shah et al., 2000). Insulin disturbances are also found in people with obesity (Cavaghan et al., 2000). Other research in the UK revealed a correlation between BMI and blood glucose (Innocent et al., 2013). Nevertheless, until today, there were so limited prove about glucagon alteration in obese people. This study aims to display the alteration pattern of glucagon in people with obesity.

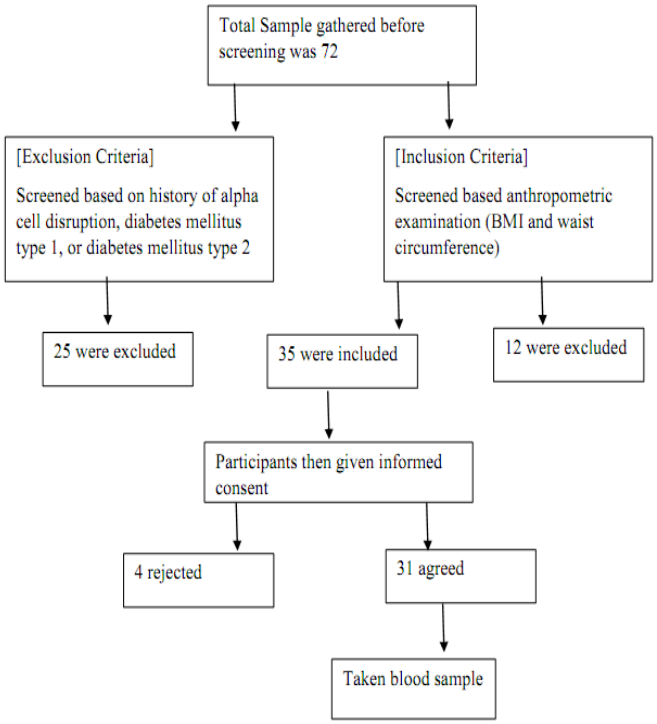
Method

The population of this study is male or female who is older than 18 years. In health care (puskesmas) Desa Sayang and Cipacing, Jatinangor, Indonesia. Inclusion criteria are BMI greater than 27 kg/m2 and waist circumference more than 102 cm for man, 88 cm for the woman. Exclusion criteria are, have been diagnosed by a doctor to suffer or proven by laboratory result to have alpha cell disruption, diabetes mellitus type 1, or diabetes mellitus type 2. This study used a descriptive quantitative design with a cross-sectional approach. Total sampling in this research followed the rule of thirty criteria. All procedures were under subjects' adequate understanding and written consent. The study used primary data from the blood samples community in Jatinangor which was then transferred to the Department of Clinic Pathology of Dr. Hasan Sadikin General Hospital.

The experts handled the blood sampling process. Then it will be measured by the ELISA method (Elab Science, USA) to determine the level of glucagon in plasma. We take the data by by cross-sectional approach. Researchers made ethical clearance and letter of permission to do the research beforehand. The ethical clearance's number is 100./UN6.C1.3.2/KPEK/PN/2016. The study variables are age, glucagon level, body weight, body height, BMI, and waist circumference. Out of 7 villages in Jatinangor, two villages Desa Sayang and Desa Cipacing were chosen randomly. The subject participants were coordinated to gather by cadres. The total samples included were 72. The next phase was screening the participants. There were two screenings, each using history taking and anthropometric examination. We asked the participants whether they had been diagnosed or proven to have alpha cell disruption, diabetes mellitus type 1, or diabetes mellitus type 2. We took 25 out of 72 based on screening results. Participants who still fulfilled the criteria were then measured based on the operational definition of obesity. The measurements of body weight, body height, and waist circumference were done by calibrated tools. It took 12 participants out of 47. Then the researcher explained the study objective, benefits the participants will have and asked for their understanding and agreement. If the participants agreed, they would get an informed consent letter. 4 of the participants rejected the consent. The remaining participants then were punctured by their veins, done by certified experts. The procedure ran in sequences. First, the participant's dominant hand was tied with a tourniquet, then the area that will be aspirated was swabbed by alcohol beforehand. The puncture used a 3cc syringe. We took the blood for about 3cc. Then the sample was placed in an EDTA tube and homogenized. These tubes were then placed in an icebox along with an

ice pack. The samples were then transferred to the Department of Clinic Pathology of Dr. Hasan Sadikin General Hospital. The samples then were handled by experts and run by an ELISA kit to determine the glucagon level of the sample.

Diagram 1. Sample Selection Procedure



Result and Discussion

The total sample included in this study was 31 then classified based on their BMI.

Table 1. Characteristic of Participants based on BMI

Characteristic	Total (n = 31)	Percentage (%)
Sex		
Female	31	100%
BMI		
27.01 – 29.99	12	39.4%
30.00 – 33.99	12	36.4%
>33.99	7	24.2%
Body Weight (kg)		
55 – 64	5	15.2%
65 – 74	15	48.5%
75 – 84	8	27.2%
>84	3	9.1%
Body Height (cm)		
135 – 144	3	9.1%
145 – 154	16	51.5%
155 – 164	11	36.4%
165 – 174	1	3.0%
Waist Circumference (cm)		
80 – 90	8	24.2%
90 – 100	11	33.3%
>100	12	42.5%

Source: Primary data, 2015

Table 2. Mean and Standard Error of Mean of Glucagon Level based on BMI

BMI group	Mean	SEM
27.01 – 29.99	158.6248333	10.03304678
30.00 – 33.99	149.9902417	11.37438632
>33.99	111.9853143	12.97802512

Source: Primary data, 2015

The research location is in Jatinangor. The participants are 100% women. This phenomenon can be explained by the data in Riskesdas 2013 mentioning there were obese females twice as many as males in Indonesia (Riset Kesehatan Dasar, 2013). Also, some theories can explain why females are more likely to be obese than males. First, women have lower physical activity than men. It causes lower energy expenditure of women (Hall, 2006). Second, estrogen is higher in females, causing weight gain in females because estrogen inhibits thyroid function and modulating the hypothalamus (Grantham and Henneberg, 2014).

Physiologically, blood glucose will be controlled in the normal range by two hormones, insulin, and glucagon. Insulin is the hormone secreted by the beta cell of the pancreas. The primary function is to maximize glucose transportation from blood to all over the body cells to be utilized then as a source of energy. When the blood glucose is too high, insulin acts in glycogenesis in the liver and skeletal muscle (Hall, 2006). On the contrary, glucagon is the hormone secreted by the alpha cell of the pancreas. The function is to increase blood glucose, through its actions in the liver. Glucagon increases glycogenolysis and gluconeogenesis in the liver (Hall, 2006; Quesada et al., 2008). In other words, insulin acts by decreasing blood glucose, while glucagon increases blood glucose levels (Hall, 2006).

Human and animal studies showed the declining trend of glucagon level and glucagon insulin ratio, both in obese mice and humans (Stern et al., 2020). Glucagon effects decrease body weight, both physiological and pathological conditions (Charron and Vuguin, 2015). Glucagon increases energy expenditure through acute and chronic thermogenesis processes (Charron and Vuguin, 2015; Kleinert et al., 2019). Exogenous Glucagon treatment could increase energy expenditure (Scott and Bloom, 2018). So the conclusion is due to lower glucagon levels, the weight-loss functions subsides and causing heavier weight and higher BMI. In people with obesity, there is chronic inflammation that relates closely to various pathogenesis of diseases. One of them is diabetes mellitus type 2. Adipose tissue in obese people secretes humoral factors, such as C-reactive protein, inflammation factors, such as monocyte-chemoattractant protein-1 (MCP-1), tumor necrosis factor-alpha (TNF alpha), and interleukin- 6 (IL-6) (Chang et al., 2015).

Insulin resistance is a condition where body tissue becomes less sensitive or even resistant to insulin (Ye, 2013). In this condition, glucose cannot be utilized optimally by body cells. Body cells cannot work well due to a lack of glucose as their energy source (Hall, 2006; Ye, 2013). If this condition lasts for some time, the body will suffer the "cell hunger phenomenon". It triggers insulin elevation to maximize glucose utilization happens through two activities. The activities are beta-cells increase and enzyme alteration. Those activities have a role in glucose metabolism in the beta-cell of the pancreas. The glucokinase role as the rate-limiting step in glucose metabolism of the beta-cell is replaced by hexokinase. Hexokinase causes elevation of insulin secretion (Moon and Won, 2015). If the insulin elevation remains constant, then the level of insulin in the blood will remain high. This condition is well known as hyperinsulinemia.

Hyperinsulinemia is defined as a stagnant condition of high insulin level in plasma in fasting. Insulin resistance is the cause of hyperinsulinemia because insulin is needed to maintain standard glucose tolerance (Cavaghan et al., 2000). Melissa K. Cavaghan, David A. Ehrmann, and Kenneth S. Polonsky stated that further work displayed increased

insulin secretion and reduced insulin clearance resulting in hyperinsulinemia. Nonetheless, hyperinsulinemia is also one of the main factors causing insulin resistance. This theory is believed to be the effect of negative feedback of the body to control the circulating insulin level. The elevated insulin level will subside the tissue's sensitivity towards insulin and causes insulin resistance (Ye, 2013).

The insulin itself has a function to regulate glucagon secretion (Hall, 2006). Along with somatostatin and amylin, insulin is the factor that inhibits glucagon secretion (Moon and Won, 2015). Researches had proved, rodents that suffer hyperglucagonemia will improve if injected with exogenous insulin (D'Alesio, 2011). With this fact, it can be stated that hyperinsulinemia will potentially inhibit glucagon secretion and causing hyperglucagonemia. Besides, resistin hormone, which is related to the development of insulin resistance, proved to be elevated in people with obesity (Al-Salam et al., 2011). Resistin causes insulin resistance, which then will aggravate hyperinsulinemia and end with glucagon inhibition.

Also, it is well known that the primary inhibitor of glucagon is the high level of blood glucose itself. In the condition where insulin resistance happens, the blood glucose will not be utilized optimally, leaving the glucose in the blood and will inhibit the glucagon secretion (Hall, 2006). Other research in the UK revealed obesity correlates with blood glucose level (Innocent et al., 2013). It will also cause inhibition of glucagon secretion, similar to our research showed. The higher the BMI, the higher the blood glucose, the lower glucagon secretion. In obese individual, glucagon and insulin ratio tend to be static in fasting and after-meal (Stern et al., 2020)

Interestingly, although diabetes mellitus type 2 is well-known because of insulin disturbance, the research done by Pankaj Shah and the team showed that there was also glucagon disturbance in people with diabetes mellitus 2. They observed hyperglucagonemia in people who suffer from diabetes mellitus type 2 (Shah et al., 2000). This research may reinforce the theory of "bihormonal hypotheses" stated by Unger and Orci back in 1975. They mentioned a combination of insulin and glucagon disturbances in the pathogenesis of diabetes mellitus type 2 (Quesada et al., 2008). Our research displays glucagon levels inversely to those in diabetes mellitus. Glucagon level tends to decrease when the BMI gets higher, opposed to the high level in diabetes mellitus type 2. Studies have been conducted to treat diabetes mellitus type 2 through glucagon receptor (Al-Massadi et al., 2019; McShane et al., 2016). Furthermore, inhibition of glucagon receptor has promising results to treat metabolic syndrome (Habegger et al., 2010). Therefore, future studies to compare glucagon and insulin treatment for obesity are needed.

Conclusion

Decrease glucagon level in the blood plasma has a potentially close relation with the increase in BMI. This glucose modulation is connected with the alteration of insulin level. The insulin might regulate glucagon secretion along with somatostatin and amylin. However, insulin modulation might also affect the homeostasis and regulation of glucagon levels in obese persons since insulin is the factor that inhibits glucagon secretion.

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