

The Pattern of Fasting and Post 75 G Glucose Loading of Glucagon-Like Peptide 1 Levels in Obese and Non-Obese Subjects

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Abstract

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BACKGROUND: Prevalence of obesity increased sharply recently; it was associated with an increased prevalence of several cardiometabolic diseases. Reduced glucagon-like peptide 1 (GLP-1) secretion is observed among obese subjects in many studies, and it may mediate the failure of insulin secretion response to food intake.

AIM: To evaluate the pattern of fasting and post 75 g glucose loading of GLP-1 levels in obese and non-obese subjects.

METHODS: An experimental study on the pattern of GLP-1 levels in fasting state and response in post 75 g glucose loading in obese and non-obese subjects, was conducted. Sixteen obese and 16 non-obese subjects were enrolled in the study, with age- and sex-matching in both groups. GLP-1 levels were measured at fasting state (0), 15, 30, 60, and 120 minutes post-glucose loading.

RESULTS: The GLP-1 response to glucose loading were similar in obese and non-obese subjects, which increased from fasting state to post glucose loading and reaching the peak levels in 15 minutes, then declined until the end of observation. There was tendency that GLP-1 levels in fasting state and post glucose loading were lower in obese subjects compared to in non-obese subjects (in fasting state, 5.67 vs. 6.16 ng/mL, $P = 0.338$; in 15 minutes, 6.20 vs. 6.94 ng/mL, $P = 0.239$; in 30 minutes 6.20 vs. 6.90 ng/mL, $P = 0.264$; in 60 minutes, 5.77 vs. 6.12 ng/mL, $P = 0.242$), but the difference were not statistically significant, except in 120 minutes (5.24 vs. 6.67 ng/mL, $P = 0.049$; in obese and non-obese subjects, respectively). Similar finding was also seen in the pattern of response (delta) of GLP-1 from time-to-time observation among obese and non-obese subjects (0-15 minutes [0.52 vs. 0.8 ng/mL, $P = 0.350$], 0-30 minutes [0.53 vs. 0.74, $P = 0.550$], 0-60 minutes [0.11 vs. 0.31 ng/mL, $P = 0.546$], in 0-120 minute [-0.42 vs. 0.31, $P = 0.006$]).

CONCLUSIONS: The patterns of GLP-1 levels post glucose loading were similar in obese and non-obese subjects which increased from fasting state to post glucose loading, reaching the peak levels in 15 minutes and then declined until the end of observation, except in non-obese subjects where the GLP-1 levels were increased at 120 minutes. There was a tendency of GLP-1 levels in fasting state and post-glucose loading to be lower in obese subjects compared within non-obese subjects.

Introduction

Based on the World Health Organization report, the prevalence of obesity has doubled between 1980 and 2008. The highest prevalence of obesity is presented in the American region (62% have overweight, and 26% are obese), and the lowest prevalence is shown in Southeast Asia (14% are overweight, and 3% experience obesity). As many as 2.8 million people die each year from being

overweight or obese [1]. Overweight and obesity trigger 44% of the incidence of diabetes, 23% of the incidence of ischemic heart disease and around 7-41% of cancer incidence [2]. The prevalence of obesity in many countries tends to be increased, including in Indonesia. Data from Basic Health Research in 2007 and 2013 reported by Ministry of Health Republic of Indonesia revealed that the prevalence of obesity in adults was increased from 13.9% to 19.7% in men and from 14.8% to 32.9% in women, and followed by increasing the prevalence of

diabetes from 5.7% to 6.9% [3], [4]. A survey on obesity and diabetes in Bali by Suastika et al., in 2011 showed that the prevalence of central obesity, impaired fasting glycemia and diabetes in adults were 35%, 13.1% and 5.9%, respectively [5].

Glucagon-like peptide-1 (GLP-1) is an incretin hormone produced by L cells in the small intestine and released in response to meal intake. Postprandial GLP-1 secretion in response to oral carbohydrate meal was reduced in obese subjects [6]. The ADDITION-PRO Study published by Faerch et al., in 2015 revealed that subjects with overweight and obesity had up to 20% reduced GLP-1 response compared with normal weight subjects. And, compared to individuals with normal glucose tolerance (NGT), women with prediabetes or type 2 diabetes mellitus (T2DM) had 25% lower GLP-1 response to an OGTT, and both in women and men with prediabetes or T2DM had 16-21% lower 120-minutes GLP-1 concentration [7]. A similar finding was seen in a study by Munoz et al., that subjects with normal weight had lower fasting GLP-1 levels significantly compared to subjects with overweight and obesity (8.27 vs 9.02 vs 8.86 pM, respectively) [8].

Impaired function of GLP-1 contributes to the impairment of insulin secretion in patients with type 2 diabetes mellitus (T2DM) [9]. A study on GLP-1 levels among normoglycemia and type 2 diabetes in Sanglah Hospital showed that fasting and 1-hour post 75 g glucose loading GLP-1 levels was lower in type 2 diabetes than in normoglycemic subjects [10].

Since there were in the variations data from several studies and there was no detailed data on the patterns of GLP-1 levels post glucose loading in obese and non-obese subjects in Indonesia, the study was conducted to evaluate the pattern of fasting and post 75 g glucose loading of GLP-1 levels in obese compared with non-obese subjects. Hopefully, the result of the study can be used to determine the peak response of GLP-1 levels post loading in the term for further study.

Methods

An experimental study on fasting and post 75 g glucose loading of GLP-1 levels among obese and non-obese subjects at Sanglah Hospital during April-June 2018 was carried out. Total of 32 subjects, 16 obese and 16 non-obese subjects was enrolled in the study and was matched for age and sex in both groups. Age of subjects was between 20-50 years (31.46 ± 4.81 years), and they had no diabetes. Subjects with obesity were confirmed if body mass index (BMI) ≥ 25 kg/m² and waist circumference (WC) ≥ 90 cm for men and ≥ 80 cm for women; and non-obese subjects were determined if BMI < 25 kg/m²

and WC < 90 for men and < 80 for women.

Blood samples for determination of plasma GLP-1 levels were drawn in fasting state (0 minutes), 15, 30, 60, and 120 minutes post 75 g glucose loading. Subjects were fasting for at least 8-12 hours before performing the procedure; after drawing blood samples in fasting state, subjects drank 75 g anhydrous glucose dissolved in 250 ml water. The plasma human GLP-1 was measured by Elisa kit with a double antibody sandwich method produced by Yanaihara Institute Inc. (multispecies specificity), Cat. No. RSCYK160R.

The study was approved by the Ethical Committee of the Faculty of Medicine, Udayana University and Sanglah Hospital (No. 2145/UN.14.2/KEP/2017), and it was authorised by the Director of Sanglah Hospital (No. LB.02.01/IXIV.2.2.1/34463/2017). All subjects were given information regarding this study and signed the informed consent. This study was conducted by the Declaration of Helsinki.

Data were expressed descriptively in mean \pm SD and analysed for normality by Shapiro-Wilk test. The difference of plasma GLP-1 levels pattern among obese and non-obese subjects was analyzed by multivariate analysis with the general linear model repeated measurement. In all statistical analyses, values of $P < 0.05$ were considered to indicate a significant difference between means.

Results

The experimental study enrolled 32 subjects consist of each 16 subjects with obesity and 16 subjects without obesity was conducted. The mean age was 31.56 years in obese subjects, and 31.37 years in non-obese subjects ($P = 0.914$), BMI was 31.10 kg/m² in obese subjects and 21.71 kg/m² in non-obese, and WC was 97.31 cm in obese subjects and 77.62 cm in non-obese subjects. Male to female ratios in obese was 8/8 and in non-obese subjects was 9/7 ($P = 0.723$) (Table 1).

Table 1: Characteristic of subjects

Variables	Obese subjects	Non-obese subjects	P
Sex (male/female ratio)	8/8	9/7	0.723
Age (years)	31.56 \pm 4.76	31.37 \pm 5.03	0.914
Height (cm)	164.40 \pm 9.89	163.25 \pm 10.16	0.747
Weight (kg)	84.74 \pm 15.94	58.08 \pm 9.10	< 0.001
Body mass index (kg/m ²)	31.10 \pm 2.91	21.71 \pm 1.69	< 0.001
Waist circumference (cm)	97.31 \pm 10.38	77.62 \pm 6.92	< 0.001

The general patterns of GLP-1 levels post glucose loading were similar in obese and non-obese subjects, who increased from fasting state to post glucose loading and reaching the peak levels in 15 minutes then declined until the end of observation, except in non-obese subjects where the GLP-1 levels

were increased at 120 minutes although it never reached the peak levels.

Table 2: GLP-1 levels in fasting state and after 75 g glucose loading in obese and non-obese subjects

GLP-1 levels (ng/mL)	Obese subjects	Non-obese subjects	P
Fasting state (0 minutes)	5.67 ± 2.14	6.16 ± 2.85	0.338
15 minutes	6.20 ± 2.21	6.94 ± 3.21	0.239
30 minutes	6.20 ± 2.19	6.90 ± 2.90	0.264
60 minutes	5.77 ± 2.29	6.12 ± 2.47	0.242
120 minutes	5.24 ± 2.04	6.67 ± 2.87	0.049

There was tendency (indicated by absolute levels) that GLP-1 levels in fasting state and post glucose loading were lower in obese subjects compared to in non-obese subjects (in fasting state, 5.67 vs. 6.16 ng/mL, $P=0.338$; in 15 minutes, 6.20 vs. 6.94 ng/mL, $P = 0.239$; in 30 minutes 6.20 vs. 6.90 ng/mL, $P = 0.264$; in 60 minutes, 5.77 vs. 6.12 ng/mL, $P = 0.242$), but the difference were not statistically significant, except in 120 minutes (5.24 vs. 6.67 ng/mL, $P = 0.049$; in obese and non-obese subjects, respectively) (Table 2 and Figure 1).

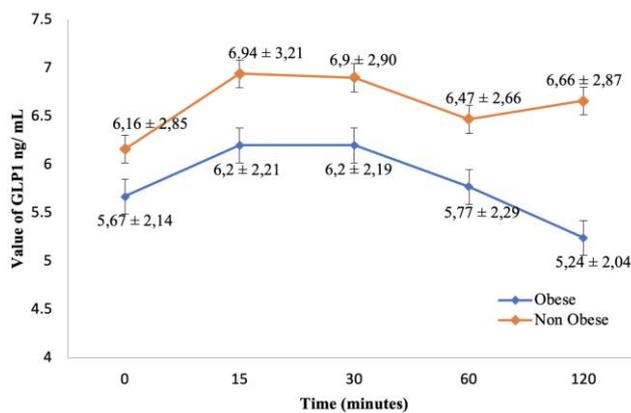


Figure 1: GLP-1 levels in fasting state and after 75 g glucose loading in obese and non-obese subjects

In this study we also compared the difference or delta (Δ) of GLP-1 levels between fasting state (0 minutes) and 15 minutes, 0 and 30 minutes, 0 and 60 minutes, and 0 and 120 minutes among obese and non-obese subjects. Similar like above finding, although obese subjects showed lower response of GLP-1 post glucose loading compared to non-obese subjects, actually there were no significant difference of Δ GLP-1 levels in 0-15 minutes (0.52 vs. 0.8 ng/mL, $P=0.350$), in 0-30 minutes (0.53 vs. 0.74, $P = 0.550$), and 0-60 minutes (0.11 vs. 0.31 ng/mL, $P = 0.546$), except in 0-120 minute (-0.42 vs. 0.31, $P = 0.006$) between obese and non-obese subjects (Table 3).

Table 3: Delta (Δ) GLP-1 levels after 75 g glucose loading in obese and non-obese subjects

Delta (Δ)GLP-1 (ng/mL)	Obese subjects	Non-obese subjects	P
Δ GLP-1 (0 – 15)	0.52 ± 0.84	0.80 ± 0.70	0.350
Δ GLP-1 (0 – 30)	0.53 ± 0.86	0.74 ± 1.12	0.550
Δ GLP-1 (0 – 60)	0.11 ± 0.97	0.31 ± 0.92	0.546
Δ GLP-1 (0 – 120)	-0.42 ± 0.56	0.31 ± 0.92	0.006

Discussion

The main objective of this study was to know the pattern of GLP-1 levels in fasting state and post-glucose loading. Our findings showed that the GLP-1 levels in fasting state and post-glucose loading were lower, but there was no significant difference, in obese compared to non-obese subjects, except at 120 minutes; and the pattern of GLP-1 levels in obese and non-obese subjects was very similar, except at 120 minutes. Peak GLP-1 levels in this study was noted in 15 minutes post-glucose loading both in obese and non-obese subjects. The response of GLP-1 measured by the delta of GLP-1 (0-15, 15-30, 30-60, 60-120 minutes) showed a similar figure with the absolute GLP-1 levels (above). In non-obese subjects, although there was increased of GLP-1 levels at 120 minutes, statistically was not different from the GLP-1 levels at 60 minutes.

The pattern is almost similar to other studies already conducted. Adam et al., in their study reported that peak levels of GLP-1 after ingestion of galactose/guar gum and standard breakfast was observed at 30 minutes and declined during observation at 60, 90, and 120 minutes. In their study there was no difference in GLP-1 levels at 0, 30, 60, 90, and 120 minutes in normal weight and obese subjects after ingestion galactose/guar gum and standard breakfast, except in 30 minutes after ingestion standard breakfast that GLP-1 levels at 30 minutes found higher in normal weight than those in obese subjects [11]. Report from Danish ADDITION-PRO study population on GLP-1 levels at 0 minutes, 30 minutes, and 120 minutes after glucose loading showed similar patterns of GLP-1 levels which increased sharply at 30 minutes from fasting state and declined at 120 minutes among 5 groups of subjects. Levels of GLP-1 at fasting state, 30 and 120 minutes were not different significantly among subjects with normal glycemic tolerance, impaired fasting glycemia (IFG), impaired glucose tolerance (IGT), IFG+IGT, and T2DM; except women with IFG+IGT and T2DM had lower GLP-1 levels at 120 minutes [7]. A study by Munoz et al. found that fasting GLP-1 in normal weight was lower than in overweight and in obese subjects, and higher fasting GLP-1 in overweight than obesity [8]. GLP-1 levels profile during OGTT in this study was similar to the observation by Manell et al., which the peak GLP-1 levels was noted at 15 minutes and declined until 120 minutes observation. Postprandial GLP-1 levels tended to be lower in adolescents with obesity, impaired glucose tolerance and T2DM compared to lean adolescents. In fasting state, GLP-1 levels in obese adolescents with normal glucose tolerance was higher compared with lean adolescents with normal glucose tolerance, adolescents with obesity and IGT and with obesity and T2DM. The finding was different in our study, and there are several studies where GLP-1 levels in fasting state among non-obese were higher than

those in obese subjects [12].

In this study, although fasting GLP-1 levels tend to be lower in obese compared to non-obese subjects, we observed no statistically significant difference. This result might be caused by the inclusion of subjects that were overweight (BMI between 23 to < 25 kg/m²) in the non-obese group; which might influence the result.

There were several studies on GLP-1 response to glucose loading among the Asian population reported. A study from Malaysia in subjects with young-onset T2DM and healthy control showed that Asian young-onset T2DM showed similar GLP-1 response to oral glucose as a control but reduced incretin effects, beta cell functions, and insulin sensitivity. The peak GLP-1 levels were observed at 15 minutes in both groups [13]. Study on GLP-1 levels during 75 g glucose loading in a Japanese population with normal glucose tolerance showed that GLP-1 levels increased post glucose loading from fasting state and the peaked levels was observed at 60 minutes and declined until the end of observation at 180 minutes [14]. Another study in Japanese by Kozawa et al., on incretin secretion in obese and non-obese subjects with T2DM and non-diabetic revealed that the peaked GLP-1 levels was observed in 30 minutes and declined until 180 minutes observation. Among the 3 groups of subjects, the peak of GLP-1 levels was tended to be higher in obese diabetic, and the lowest in a non-obese diabetic. The two finding from Japanese studies is different from our study and other studies which the peaked GLP-1 levels were found at 15 minutes [15].

In general it can be concluded that pattern of GLP-1 levels is similar in subjects with lean normoglycemia, obesity, and glucose intolerance which increased post glucose loading and achieving the peak levels at around 15-30 minutes and declining thereafter until 120-180 minutes of observation; but normal or lean subjects have higher GLP-1 levels than those with obesity, prediabetes or T2DM. If we compare between our study and other studies, especially in Caucasians, increasing response of GLP-1 at 15 minutes post-glucose loading from the fasting state in our study was not as steep as other studies have observed. We do not know the certain reasons, but we assume that the blunted response of GLP-1 may cause T2DM earlier in the Indonesian population. We also could not explain the phenomenon of increased GLP-1 levels at 120 minutes in non-obese subjects in this study.

In conclusion, the patterns of GLP-1 levels post glucose loading were similar in obese and non-obese subjects, which increased from fasting state to post glucose loading and reaching the peak levels at 15 minutes and then declining until the end of observation, except in non-obese subjects where GLP-1 levels were increased in 120 minutes. There was a tendency of lower GLP-1 levels in fasting state

and post-glucose loading in obese subjects compared to non-obese subjects.

Authors' Contributions

IBAN design the study and performed data analysis, interpreted the data, and drafted the manuscript. MRS and KS participated in the design of the study and helped revise the final manuscript. All authors read and approved the final manuscript.

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