Similar Blood Glucose Pattern with Highest Peak at Minute 45 on Oral Glucose Tolerance Test Despite Higher Fasting Insulin and Insulin Resistance in Healthy Obese than Non-Obese Subject

Made Ratna Saraswati[1], Ida Bagus Aditya Nugraha[2], Ketut Suastika[1]*

[1]Division of Endocrinology and Metabolism, Department of Internal Medicine, Faculty of Medicine Udayana University - Sanglah Hospital, Denpasar, Bali, Indonesia.
[2]Department of Internal Medicine, Faculty of Medicine Udayana University - Sanglah Hospital, Denpasar Bali, Indonesia.

**** Corresponding Author:***
Made Ratna Saraswati, MD. Division of Endocrinology and Metabolism, Department of Internal Medicine, Faculty of Medicine Udayana University - Sanglah Hospital. Jl. P.B. Sudirman, Kota Denpasar, Bali 80232, Indonesia. Email: *ratnasaraswati@unud.ac.id*

ABSTRACT

Background*:** *Obesity increase the risk for type 2 diabetes through induction of insulin resistance. Diagnosis of diabetes were based on blood glucose level. However, insulin resistance may had happened far before diagnosis itself. This study aimed to compare fasting insulin level, insulin resistance, and blood glucose pattern during oral glucose load in healthy obese and non-obese subject.* ***Methods: *This semi-experimental study was conducted at Department of Internal Medicine, Sanglah Hospital, Denpasar. Sixteen subjects in each obese and non-obese group were matched by age and sex. Obesity was defined based on body mass index (BMI) of ≥25kg/m^2 and waist circumference (WC) ≥80cm (female) or ≥90cm (male). The non-obese group was defined by BMI of 18-25kg/m^2 and WC <80cm (female) or <90cm (male). Fasting insulin level and blood glucose was measured at minute 0, 15, 30, 45, 60, 75, 90, 120 after glucose load of 75 grams. Insulin resistance was calculated based on homeostasis model assessment of insulin resistance (HOMA-IR) with the following formula: HOMA-IR = (FPI×FPG)/22.5. Normal glucose tolerance (NGT) and impaired glucose tolerance (IGT) subject was defined by American Diabetes Association (ADA) criteria.* ***Results*:** *Fasting insulin level in obese subjects was higher than non-obese subjects with median 12.75 (range 3.70 – 41.30) vs 3.80 (1.80 – 36.80) µU/mL, p=0.041. HOMA IR was also higher in obese subjects compared to non-obese subjects: 2.45 (0.70 – 8.00) vs 0.80 (0.40 – 8.50), p=0.001. Fasting insulin level was correlated with BMI (r=0.559, p=0.001) and WC (r=0.633, p<0.001). A significant correlation was also detected between HOMA IR with BMI (r=0.528, p=0.002) and WC (r=0.600, p<0.001). Blood glucose pattern in four groups: obese IGT, obese NGT, non-obese IGT, and non-obese NGT, were typically similar, in particular two peaks of blood glucose. The first peak was the highest blood glucose, shown in minute 45 in both obese and non-obese subjects. The second peak was lower than the first peak, found in minute 75 among NGT and minute 90 among IGT subject. Blood glucose level for each measurement point was consistently higher in obese than non-obese subjects.* ***Conclusion*:** *Fasting insulin level and HOMA-IR were higher in obese than in non-obese subjects. BMI and WC were significantly correlated with fasting insulin level and HOMA IR, so that high BMI and WC can be an earlier clinical sign of insulin resistance and prediabetes. Pattern of blood glucose level after oral glucose load were similar with two peaks, and blood glucose consistently higher in obese compared to non-obese subjects. The highest peak of blood glucose, shown in minute 45 in both obese and non-obese subjects.*

Keywords: *Blood glucose pattern, OGTT, fasting insulin, HOMA IR*

INTRODUCTION

Prevalence of diabetes is markedly increasing, along with increasing prevalence of obesity. According to the IDF Atlas 10th edition 2021, the number of people with diabetes in Indonesia is 19.6 million, placing at the 5th rank of Top 10 countries or territories for number of adults (20–79 years) with diabetes in 2021 and 2045, rising from 7th rank in 2019.[1,2] The increasing rank is predictable since Indonesia was at 3rd rank of top 10 countries or territories for the number of adults (20–79 years) with impaired glucose tolerance (IGT) in 2019.

In order to avoid burden of diabetes and its complications, preventing diabetes is important. Early detection and early management of prediabetes is the key strategy to prevent diabetes. Study on 3234 nondiabetic persons aged 25 or more with elevated fasting and post-load plasma glucose concentrations revealed that a lifestyle-modification program with the goals of at least a 7 percent weight loss and at least 150 minutes of physical activity per week in Diabetes Prevention Program, reduced the incidence of diabetes by 58 percent after followed for an average of 2.8 years.[3] Similar study on long term effect of lifestyle interventions to prevent diabetes in the China Da Qing Diabetes Prevention Study enrolled 577 adults with impaired glucose tolerance showed 51% lower incidence of diabetes during the active intervention period and a 43% lower incidence over the 20 year period.[4]

Obesity increased the risk for type 2 diabetes through induction of insulin resistance which is thought to precede the development of diabetes 10 to 15 years.[5-7] The development of insulin resistance typically results in a compensatory increase in endogenous insulin production, and at the time of compensatory insulin secretion, blood sugar may remain normal while hyperinsulinemia as a consequences of insulin resistance affect the metabolism known as metabolic syndrome.[8] Direct measurement of insulin resistance can be done by measuring insulin-mediated glucose disposal during insulin suppression test and the gold standard is the hyperinsulinemic-euglycemic glucose clamp technique. This test is a research technique with limited clinical applicability due to impractically and expensive. Some efforts have been tried to use both the fasting plasma glucose and insulin concentration to estimate of insulin-mediated glucose disposal in more practical way, and several indices have been proposed, including homeostasis model assessment insulin resistance (HOMA IR).[9]

In a study of 490−healthy nondiabetic volunteers, fasting plasma insulin concentration accounted for approximately one-third of the variability in insulin-mediated glucose disposal, and fasting plasma insulin concentration as well as HOMA IR was significantly correlated to the specific estimate of insulin action.[10] These two approaches, measuring fasting plasma insulin level and calculating HOMA IR, has almost universally been used in large population-based epidemiological studies, however in clinical practice still relatively expensive and not widely available. For a clinical purpose, we need to define more practical surrogate marker of insulin resistance.

There are some difficulties in early detection of diabetes and prediabetes since the symptoms may not be obvious. The available test in clinical practice as per guideline are fasting blood glucose and blood glucose two hours post oral glucose load during oral glucose tolerance test (OGTT), which may lead particular high risk patient detected as normal glucose tolerance since the peak blood glucose could not be captured. This study aims to compare the fasting insulin level, insulin resistance, and blood glucose pattern during oral glucose load in healthy subject without history of diabetes divided into obese and non-obese groups. This study defined glucose pattern in more detail during OGTT and confirmed the importance of simple measurement of obesity in clinical practice to recognize insulin resistance regardless blood sugar level.

METHODS

This semi-experimental study was conducted at Department of Internal Medicine, Sanglah Hospital, Denpasar. A total of 32 subjects without history of diabetes were recruited, grouped into obese and non-obese groups, matched by age and sex. Age of subjects was between 20-50

years (mean age 31.46 SD 4.81 years). Obesity was defined based on body mass index (BMI) of ≥25kg/m^2 and waist circumference (WC) ≥80cm (female) or ≥90cm (male). The non-obese group had BMI of 18-25kg/m^2 and WC <80cm (female) or <90cm (male).[11]

Subjects were instructed to fast for at least 8 and maximum 12 hours before performing the procedure. Blood sample for measurement of fasting plasma glucose level and fasting insulin level were drawn in fasting state (minute 0). Oral glucose load using 75 g anhydrous glucose dissolved in 250 ml water was done in no more than 5 minutes. During oral glucose tolerance test (OGTT), capillary blood glucose level were measured in several time points at minutes 0, 15, 30, 45, 60, 75, 90 and at 120 after glucose load. Another blood sample was drawn at minute 120 during OGTT for plasma glucose to confirm the normal glucose tolerance (NGT), impaired glucose tolerance (IGT), or diabetes categorization.

Fasting insulin level was measured by solid phase enzyme-labeled chemiluminescent immunometric assay, using Immulite® 2000 Insulin analysis system, Cat. No. L2KIN2 (Siemens). Fasting plasma glucose (FPG) and 2 hours plasma glucose (2hPG) during oral glucose tolerance test (OGTT) were measured by enzymatic hexokinase method. Capillary blood glucose was measured using glucometer (Accucheck®, Roche) at minutes 0, 15, 30, 45, 60, 75, 90, and 120 after 75-gram glucose load.

Based on FPG and 2hPG during OGTT, subjects were categorized as normal, prediabetes, or diabetes. Prediabetes are defined by impaired glucose tolerance (IGT) and/or impaired fasting glucose (IFG). IFG is defined as FPG levels between 100-125mg/dL, and IGT as 2-h PG levels between 140 and 199 mg/dL during 75-g OGTT. Diabetes is defined by FPG ≥126mg/dL or 2-h PG ≥200 mg/dL during OGTT.[12] Insulin resistance was calculated based on homeostasis model assessment of insulin resistance (HOMA-IR) with the following formula: HOMA-IR=(FPI×FPG)/22.5.[13]

Data were analyzed for normality by Shapiro-Wilk test, and expressed descriptively in mean ± SD for normally distributed data or in median with range for not normally distributed data. The difference of median fasting insulin and HOMA IR were calculated by non-parametric test. Correlation between fasting insulin and HOMA IR with BMI and WC were measured by Spearman's rho. Repeated measurement general linier model was applied to define the blood glucose pattern during OGTT. In all statistical analyses, values of $p<0.05$ were considered significant.

The study approved by the Ethical Committee of the Faculty of Medicine Udayana University and Sanglah Hospital (No. 2145/UN.14.2/KEEP/2017), and 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.

RESULTS

Subject recruited in this study aged between 27-48 years old (mean 31.46 + 4.81), age and sex matched, each group consisted of 8 males and 8 females. Based on the FPG and 2-h PG during OGTT, 4 subjects in obese group and 1 subject in non-obese group were categorized IGT, none of the subjects were categorized IFG or diabetes. Distribution of fasting insulin level and HOMA-IR were not normally distributed. Thus, data for these variables was presented in median and range (**Table 1**).

Fasting insulin in obese subjects was higher than non-obese subjects, median 12.75 (range 3.70 – 41.30) vs 3.80 (1.80 – 36.80) μU/mL, p=0.041. HOMA IR was also higher in obese subjects than non-obese subjects: 2.45 (0.70 – 8.00) vs 0.80 (0.40 – 8.50), p=0.001 (Table 1). Fasting insulin level was correlated with BMI (r=0.559, p=0.001) and WC (r=0.633, p<0.001). A significant correlation was also detected between HOMA IR with BMI (r=0.528, p=0.002) and WC (r=0.600, p<0.001).

The pattern of increasing blood glucose level during OGTT was similar between obese and non-obese subjects. Pattern in both groups showed two peaks. The first peak blood glucose was at minute 45 (161.31 SD 25.24 mg/dL) follow by slightly lower at minute 60, and second

Table 1. Characteristics of the Subject.

Variable (unit)	Data distribution mean (SD) or median (range)		
	Obese N=16	Non obese N=16	Total N=32
Age (years)	31.56 (4.76)	31.37 (5.03)	31.46 (4.81)
Body height (cm)	164.40 (9.89)	163.25 (10.16)	163.82 (9.88)
Body weight (kg)	84.74 (15.94)	59.33 (10.77)	72 (18.59)
BMI (kg/m^2)	31.10 (2.91)	22.15 (2.31)	26.62 (5.23)
WC (cm)	97.31 (10.38)	77.62 (6.92)	87.46 (13.24)
Fasting insuln (µU/mL)	12.75 (3.70 – 41.30)	3.80 (1.80 – 36.80)	7.25 (1.80 – 41.30)
HOMA IR	2.45 (0.70 – 8.00)	0.80 (0.40 – 8.50)	2.50 (0.40 – 8.50)

peak was at minute 75 (155 SD 26.23 mg/dL) followed by lower level of blood glucose the minutes after (**Figure 1**). Blood glucose level for each point of measurement was consistently higher in obese than non-obese subjects. Test of sphericity for the blood glucose is significant (p=0.000) so the assumption of sphericity has not been met, then tests of between subject effects was conducted using the Greenhouse-Geisser row which was significant (p= 0.000).

Since not all subjects were categorized NGT based on the FPG and 2-h PG during OGTT (4 subjects in obese group and 1 subject in non-obese group were categorized IGT), the pattern of blood glucose during OGTT were further divided into four groups, obese IGT, obese NGT, non-obese IGT, and non-obese NGT. Generally, the blood glucose pattern in IGT were higher than NGT in all point minutes of examination, in both obese and non-obese groups. Blood glucose pattern in these 4 groups were typically similar, showing two peaks of blood glucose level. The first peak of blood sugar was in minute 45 in all groups, the second peak among NGT subjects was found at minute 75 meanwhile among IGT subjects was found delayed: at minute 90 (**Figure 2**).

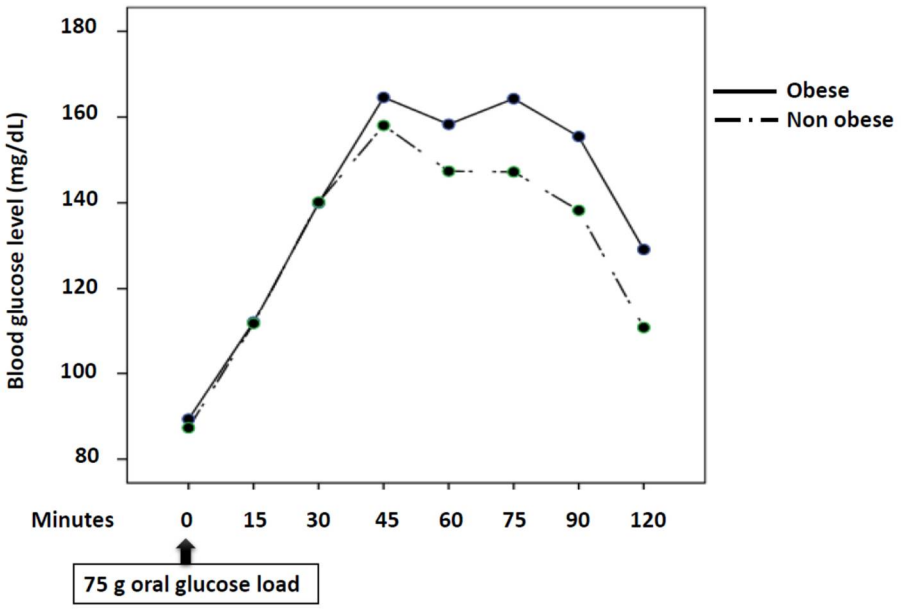

Figure 1. Blood glucose level for each minute point measurement during oral glucose tolerance test (OGTT) in obese and non-obese

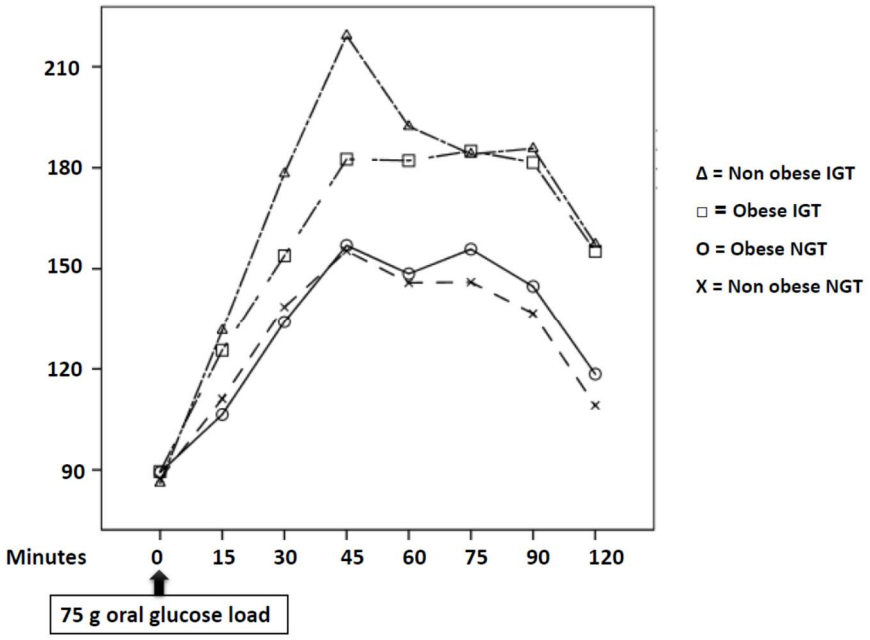

Figure 2. Blood glucose level for each minute point measurement during OGTT in 4 groups: obese IGT, obese NGT, non-obese IGT, and non-obese NGT
(OGTT = oral glucose tolerance test, NGT= normal glucose tolerance, IGT=impaired glucose tolerance)

Based on Kruskal-Wallis test, the distribution of fasting insulin across NGT or IGT and obese or non-obese categorizations were significantly different (p=0.005). The median of fasting insulin level across these categories were significantly different (p=0.41) (**Figure 3a**).

Similar finding was found for the distribution of HOMA IR between NGT or IGT categories in both obese and non-obese groups (p=0.004). The median of HOMA IR across these categories were significantly different (p=0.001) (**Table 2**). Since there was only 1 non-obese subject with IGT, the median data and range has been omitted. Fasting insulin level in this subject was 1.90 µU/mL and HOMA IR was 0.4. One non-obese subject with NGT has outlier fasting insulin level (36.80 µU/mL) and HOMA IR (8.5), as shown on the figure (**Figure 3a** and **3b**). Fasting insulin and HOMA IR were not correlated with blood glucose level at any of point measurement between minute 15 to 120.

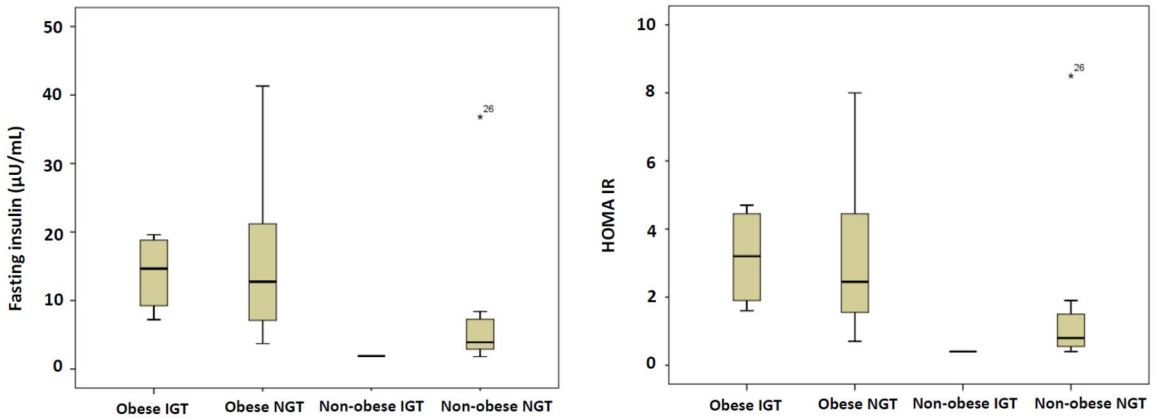

Figure 3. Median fasting insulin (a) and HOMA IR (b) in each group of obese IGT/NGT and non-obese IGT/NGT
(NGT= normal glucose tolerance, IGT=impaired glucose tolerance)

Tabel 2. Fasting Insulin and HOMA IR in Obese and Non-obese Subject.

Group		Fasting insulin ((µU/mL)		HOMA IR	
		Median	Range	Median	Range
Obese	IGT	14.65	7.20 – 19.60	3.20	1.60 – 4.70
	NGT	12.75	3.70 – 41.30	2.45	0.70 – 8.00
Non obese	NGT	3.90	1.80 – 36.80	0.80	0.40 – 8.50

DISCUSSION

Obesity is a rapidly growing nutritional disorder characterized by excessive accumulation of adipose tissue. Increased body weight is associated with insulin resistance and type 2 diabetes mellitus. In this study we found that obese subjects have higher fasting insulin level as well as HOMA IR, and both the fasting insulin level and HOMA IR were correlated significantly with the clinical obese parameters: BMI and WC. Higher fasting insulin level and HOMA IR, as well as positive correlation between BMI dan WC with HOMA IR confirmed the hypothesis that obese subjects were more likely to have insulin resistance compared with non-obese subjects.

In this study, one non-obese subject with NGT has high level of fasting insulin (36.80 µU/mL) and HOMA IR (8.5), as shown on the figure (**Figure 3a** and **3b**). Insulin resistance is present in the majority of patient with impaired glucose tolerance (IGT) or non-insulin-dependent diabetes mellitus (NIDDM) and in 25% of nonobese with normal oral glucose tolerance.[14]

Based on history, all subjects were considered to be healthy. However based on 2-hour plasma glucose during OGTT, 4 out of 16 subjects (25%) in obese group were identified as prediabetes (more specifically IGT), while in non-obese group only 1 subject was categorized as such (6.3%). This finding confirmed that obese subjects were more prone to be prediabetes. Blood glucose level for each point of measurement was consistently higher in obese than non-obese subjects. Pattern of increasing blood glucose level during OGTT were similar between obese and non-obese subjects in which they shown 2 peaks of blood glucose level regardless of their categorization as NGT or IGT. The first peak level of blood glucose level occurred in minute 45 during OGTT, followed by lower blood glucose. The second peak occurred in minute 75 among NGT subjects and a little delayed in minute 90 among IGT subjects. Since the number of IGT subjects was low, we need further study to evaluate the pattern of blood glucose in IGT subjects and evaluate the insulin response to explain this finding.

Fasting and 2-hour after 75 g glucose load on OGTT have been used to diagnose prediabetes and diabetes. However, evidence indicates that clinically relevant pathophysiological information can be obtained by adding intermediate time-points to a standard OGTT. A population-based study of 3666 Asian Indians underwent a three-point (fasting, minute 30 and 120) OGTT at baseline and then followed by another OGTT after 2 years follow up, found that elevated blood glucose at minute 30 after glucose load was associated with high risk of incident diabetes, even in individuals classified as NGT by a traditional OGTT.[15] Another cohort study of 5861 participants without diabetes at baseline from the Danish Inter99 study underwent similar three point (fasting, minute 30 and 120) OGTT identified four distinct glucose patterns during the OGTT, and the pattern with elevated glucose at minute 30 was associated with increased risk of diabetes and all-cause mortality rate independent of fasting and 2 hours glucose levels.[16] There is no data of minute 45 blood glucose in these particular studies. These findings alert us to define the highest peak of blood glucose during OGTT, so that we could choose the best time reflecting peak of blood glucose. In our study by doing frequent blood glucose measurement every 15 or 30 minutes (minute 0, 15, 30, 45, 60, 75, 90, 120) after glucose load on OGTT we found two peaks of the glucose pattern. Compare to these two studies, instead of minute 30, we found the first peak was at minute 45, the second peak was at minute 75 among NGT subject and at

minute 90 among IGT subjects. The first peak at minute 45 was the highest. Based on our finding, we conclude that considering only fasting and 2 hours glucose levels during an OGTT may not revealed the actual highest level of blood sugar. We suggest to check blood glucose at minute 45 during OGTT, in particular patient who are obese and high risk diabetes. Whether the 45 minute blood glucose provide a better prediction for future diabetes need further evaluation. We suggest to follow the obese subject group and reevaluate the blood glucose and fasting insulin level after a period of time. The data would be more comprehensive and important, if we could prescribe interventions to prevent more severe insulin resistance followed by longitudinal evaluation whether such intervention could prevent prediabetes and diabetes.

CONCLUSION

Fasting insulin level and HOMA-IR were higher in obese than in non-obese subjects. The BMI and WC were significantly correlated with fasting insulin level and HOMA IR. Since fasting insulin and HOMA IR were not correlated with blood glucose level during OGTT, these two simple markers of clinical obesity (high BMI and WC) should be taken as an earlier predictor of insulin resistance and prediabetes in clinical practice regardless blood glucose level.

Pattern of blood glucose level after oral glucose load were similar with two peaks despite higher fasting insulin and HOMA IR in obese than in non-obese subject. Even though fasting insulin and HOMA IR were not correlated with blood glucose level at any of point measurement during OGTT, blood glucose consistently higher in obese compared to non-obese subjects. The highest peak blood glucose, shown in minute 45 in both obese and non-obese subjects. Based on this finding, we suggest to check blood glucose at minute 45 during OGTT in particular for obese and high risk diabetes patient.

AUTHORS' CONTRIBUTION

MRS designed the study and performed data analysis, interpreted the data, and drafted the original manuscript. IBAN participated in informing subject and data collection. KS helped revised the final manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

Laboratory measurement was conducted at Prodia Laboratory, Denpasar, and the authors would like to convey gratitude for the technical assistance provided by the laboratory. We would also like to convey our gratitude to Roche for providing the glucometer (Accucheck®, Roche) used in capillary blood glucose measurement in this study.

REFERENCES

1. International Diabetes Federation. IDF Diabetes Atlas, 10th edn. Brussels, Belgium: 2021. Available at: https://www.diabetesatlas.org. Downloaded: 20 December 2021
2. IDF Diabetes Atlas. 9th Edition, 2019. https://www.diabetesatlas.org/upload/resources/2019/IDF_Atlas_9th_Edition_2019.pdf. Downloaded: 20 December 2021.
3. Knowler WC, Barrett-Connor E, Fowler SE, et al. Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002;346(6):393-403. doi: 10.1056/NEJMoa012512. PMID: 11832527; PMCID: PMC1370926.
4. Pendergrass M, Li G, Zhang P, et al. The long-term effect of lifestyle interventions to prevent diabetes in the China Da Qing Diabetes Prevention Study: a 20-year follow-up study. Diabetes Care. 2008;31:1921-2.
5. Blüher M. Adipose Tissue dysfunction in obesity. Exp Clin Endocrinol Diabetes. 2009;117(06):241-50. doi:10.1055/s-0029-1192044.
6. Ye J. Mechanisms of insulin resistance in obesity. Front Med. 2013;7(1):14-24. doi:10.1007/s11684-013-0262-6.
7. Kahn BB, Flier JS. Obesity and insulin resistance. J Clin Invest. 2000;106(4):473-81. doi:10.1172/JCI10842.
8. Reaven G. The metabolic syndrome or the insulin resistance syndrome? Different names, different concepts, and different goals. Endocrinol Metab Clin N Am. 2004;33:283–303.

9. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Tu rner RC. Homeostasis model assessment: insulin resistance and b-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412–9.
10. Yeni-Komshian H, Carantoni M, Abbasi F, Reaven GM. Relationship between several surrogate estimates of insulin resistance and quantification of insulin-mediated glucose disposal in 490 healthy, nondiabetic volunteers. Diabetes Care. 2000;23:171–5.
11. World Health Organization. Regional Office for the Western Pacific. (2000). The Asia-Pacific perspective: redefining obesity and its treatment. Sydney : Health Communications Australia. https://apps.who.int/iris/handle/10665/206936.
12. American Diabetes Association. 2. Classification and diagnosis of diabetes: Standards of Medical Care in Diabetes – 2021. Diabetes Care. 2021;44(Suppl.1):S15-S33.
13. Diabetes trial units. HOMA calculator. The Oxford Centre for Diabetes, Endocrinology and Metabolisms, University of Oxford. Available from: http://www.dtu.ox.ac.uk/homacalculator/. Downloaded: 10 August 2020.
14. Reaven GM. Role of insulin resistance in human disease. Diabetes. 1988;37(12):1595-607. Doi:10.2337/diab.37.12.1595.
15. Hulman A, Gujral UP, Narayan KMV, Pradeepa R, Mohan D, Anjana RM, Mohan V, Færch K, Witte DR. Glucose patterns during the OGTT and risk of future diabetes in an urban Indian population: The CARRS study. Diabetes Res Clin Pract. 2017;126:192-197. doi: 10.1016/j.diabres.2017.01.009. Epub 2017 Feb 17. PMID: 28259008; PMCID: PMC5408861.
16. Hulman A, Vistisen D, Glümer C, Bergman M, Witte DR, Færch K. Glucose patterns during an oral glucose tolerance test and associations with future diabetes, cardiovascular disease and all-cause mortality rate. Diabetologia. 2018;61(1):101-107. doi: 10.1007/s00125-017-4468-z. Epub 2017 Oct 6. PMID: 28983719.