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Original Research Paper

INSULIN AND GLUCOSE PLASMA LEVELS IN ORAL GLUCOSE TOLERANCE TEST

Diabetology

KEYWORDS: glucose tolerance, diabetes, insulin, glucose tolerance test, reference ranges

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ABSTRACT

Glucose, insulin and glycated hemoglobin levels in oral glucose tolerance test (OGTT) are the basis for the diagnosis of insulin resistance, pre-diabetes, type 1 and type 2 diabetes mellitus. Both in Europe and the United States, the accepted values for the reference range of glucose and insulin in OGTT change every few years. Glucose and insulin levels at 60 and 120 minutes are the most widely used plasma biomarkers, regardless of the new biomarkers and indices, such as hyperinsulinemic-euglycemic clamp, HbA1c, HOMA-IR, etc. In this study, insulin and glucose levels were monitored in 100 clinically healthy controls. The obtained results are discussed on the background of the available new literature data on glucose and insulin.

INTRODUCTION: Glucose is the main energy source of the human body. The hormone insulin is synthesized by the beta-cells of the pancreatic islets, and then enters into the blood and regulates the absorption of glucose by the cells. Hyperglycemia occurs in cases of inadequate insulin production or insulin resistance (IR), when the cells of the organism do not respond to insulin effects. It is assumed that determination of fasting glucose and insulin levels is not sufficiently informative and therefore, their dynamic monitoring after oral ingestion of 75 g glucose (OGTT) is suggested. There are various suggestions for monitoring glucose and insulin levels – at 0 and 30, 60, 120 minutes, etc., after ingestion of glucose solution. In this study, we monitored glucose and insulin levels in the fasting state, and then at 60 and 120 minutes after ingestion of 75 g glucose, to establish our original reference ranges in OGTT. Peak glucose and insulin levels were registered at 60 minutes, and after then these decreased. The increase over baseline levels was different for the two biomarkers.

MATERIAL AND METHODS: The study involved 100 clinically healthy controls, 43 men and 57 women, aged 17-78 years. In all subjects, glucose and insulin levels were studied in the morning, after a 12-hour fasting period. Then each subject was given a solution containing 75 g glucose. After ingestion of the glucose solution, glucose and insulin levels were determined exactly at 60 and 120 minutes. Glucose levels were determined in the serum by using the hexokinase method (Abbott Diagnostics, Architect c8000), while those of insulin by using CLIA (Advia Centaur XP, Siemens Healthcare). All participants were subjected to clinical examination, urine analysis, blood count and main biochemical tests. The data were processed by the statistical program REFVAL.

RESULTS: The obtained results are presented in Tables 1 and 2. Fasting glucose levels ranged from 3.64 to 6.14 mmol/l with X_{mean} of 4.89 ± 0.61, those of insulin from 3.87 μU/ml to 29.88 uU/ml with X_{mean} of 13.40 ± 7.40. After conducting the OGTT, glucose levels expectedly increased (X_{mean} 7.01 ± 2.61) at 60 minutes and slightly decreased at 120 minutes compared to those at 60 minutes (X_{mean}5.35 ± 1.45). At 60 minutes, insulin levels also increased to X_{mean} of 93.72 ± 66.75 and at 120 minutes decreased to those at 60 minutes with X_{mean} of 49.15 ± 51.24. Mean blood glucose and insulin levels increased significantly ($p<0.05$) in the next 60 and 120 minutes. At 60 and 120 minutes glucose levels increased 1.45 and 1.07 times, and those of insulin 6.99 and 3.66 times from the baseline, respectively. Maximum glucose and insulin levels were registered at 60 minutes and then decreased, but still not reaching the zero baseline level.

Table 1. Glucose and insulin levels in OGTT

Parameter	Minimal value	Maximal value	X_{mean}	SD
Glucose mmol/l				
0 min.	3,64	6,14	4,98	0,61
60min	3,60	13,11	7,01	2,61
120 min	3,20	8,64	5,35	1,45
Insulin μU/ml				
0 min	3,87	29,88	13,41	7,41
60 min	18,72	269,00	93,72	66,75
120 min	4,44	212,00	49,15	51,24

Table 2. Ratios between the results obtained at 0, 60 and 120 minutes

Ratios between the results obtained at 0, 60 and 120 minutes						
Calculated value	Glucose			Insulin		
	60/0 min	120/60 min	60/120 min	60/0 min	120/60 min	60/120 min
Mean	1,407	0,763	1,310	6,989	0,529	1,907
Min	0,661	0,336	0,591	0,548	0,085	0,271
Max	2,444	1,692	2,978	2,978	3,697	11,807
SD	0,427	0,272	0,439	0,439	0,512	2,625
CV %	30,730	32,530	33,070	59,970	85,762	85,150

DISCUSSION: For a long time, the diagnosis of insulin resistance (IR), pre-diabetes (PD) and type 1 and type 2 diabetes mellitus (DM1 and DM2) was based on the determination of glucose levels in the fasting state or in dynamics during the OGTT. Later on, the determination of insulin levels was also included, because the latter can predict the onset of diabetes, distinguish DM2 from autoimmune diabetes in adults, etc. (1,2,3). Recently, due to the high correlation (r = 0.685) between glucose and HbA1c levels, the WHO discussed the latter as a possible standard biomarker (3,4,5). According to the American Diabetes Association (ADA), glycated hemoglobin (HbA1c) may replace the fasting blood glucose for the diagnosis of the indicated diseases. HbA1c is an important indicator for the long-term glycemic control, making possible to reflect the cumulative glycemic history in the past two to three months. HbA1c not only provides a reliable measure of chronic hyperglycemia, but also correlates well with the risk of long-term heart complications in diabetes. Elevated HbA1c is also seen as an independent risk factor for coronary heart disease and stroke in patients with or without diabetes (1,5,6). ***Substituting glucose by HbA1c is still not accepted by all health professionals because of methodological and financial***

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reasons, and changes in glycated hemoglobin levels in anemia and in some children. It is rather required to determine, as accurately as possible, the reference ranges of glucose and insulin in the fasting state and after OGTT, because there is no 100% correspondence between the changes in glucose levels and OGTT. Glucose levels are also affected by the preceding three-day diet. The cut-off of these indicators is still disputable. The reference values of these indicators continue to be discussed and changed every few years (1,7,8,9,10). The determination of fasting glucose and insulin is not sufficiently informative for the diagnosis of IR, PD, DM1 and DM2 and therefore, the inclusion of OGTT has been suggested. Early and accurate diagnosis of these diseases is very important because globally, there is a tendency for their continued increase, reaching to epidemic. The informative value of fasting insulin is still argued and therefore, it has been suggested fasting insulin and glucose to be monitored simultaneously and dynamically in OGTT (9,10,11). The use of the so-called "Gold standard" (hyperinsulinemic-euglycemic clamp) is difficult to achieve under normal conditions. Dynamic monitoring of the indices in OGTT is performed at various intervals and durations – at 30 minutes or 1 hour to the 1st, 2nd, 3rd or 4th hours. Sufficiently informative are considered, however, the levels at 0, 60 and 120 minutes, and according to a number of guidelines – these at 0 and 120 minutes are completely sufficient (11,12,13,14,15). Therefore, we studied glucose and insulin levels at 0, 60 and 120 minutes in 100 clinically healthy controls to establish our original reference values. The ADA and the WHO (2,5,6,16) recommend different glucose levels for the diagnosis of diabetes (Table 3). Thus, fasting glucose levels less than 5.6 mmol/l are considered normal by the ADA, and should be less than 6.1 mmol/l according to the WHO; for impaired glucose tolerance (pre-diabetes), these should be higher than 5.6 mmol/l and less than 6.90 mmol/l according to the ADA, and higher than 6.1 mmol/l and less than 7.0 mmol/l according to the WHO; for diabetes mellitus, these should be higher than 7.0 mmol/l and higher than 7.1 mmol/l according to the ADA and the WHO, respectively. In OGTT, both the ADA and the WHO accept for normal glucose levels <7.8 mmol/l, >7.8 mmol/l and <11.0 mmol/l for impaired glucose tolerance and >11.1 mmol/l for diabetes. In OGTT of healthy controls, glucose levels are highest at 60 minutes and then decrease.

Table 3 Glucose and HBA1c according to WHO and ADA

	Glucose (mmol/)	
	WHO 2015	ADA 2015
Health		
Glucose 0 min.	<6.1	<5.6
Glucose 120 min.	<7.8 l	<7.8
HbA1c	< 5.6 %	< 5.4%
Pre-Diabetes		
Glucose 0 min.	6.1–7.0	5.6 – 6.9
Glucose 120 min.	7.8-11.0	7.8 – 11.0
HbA1c	5.7 – 6.4	5.5 – 6.4 %
Diabetes		
Glucose 0 min.	>7.1	> 7.0
Glucose 120 min.	> 11.1	> 11.1
HbA1c	> 6.5 %	> 6.5%

A random plasma glucose level in a healthy individual should not exceed 7-8 mmol/l. Moreover, glucose levels in the serum are 1.15% lower than in the plasma, and 5% lower in the heparinized plasma with morning levels higher than these in the afternoon. For hypoglycemia, glucose levels less than 3.0-3.5 mmol/l are accepted (7). In our controls, fasting glucose levels were X = 4.98 + 0.85 and X = 7.01 + 2.61 at 60 minutes and X_{mean} 5.35 ± 1.45 at 120 minutes in OGTT. Peak glucose levels were registered at 60 minutes and then these started to decrease, but still not reaching the baseline levels up to 120 minutes. Glucose levels correlated well with the age and BMI and less with the gender. With regard to insulin concentrations, data are much less and disputable. There is no argue that insulin concentrations in the fasting state and in dynamics in OGTT are also essential for the diagnosis of IR, PD and DM (17,18,19). However, there is still no consensus on the ideal level in the fasting state and in OGTT (7,12). It is assumed that a definite insulin value should be registered in the fasting state. Most commonly 5-8 or 10-20 μU/ml are reported. In the United States, insulin values of 8.8 μU/ml for men and 8.4 μU/ml for women are accepted, while other authors believe that the ideal level is below 8.4 μU/ml or within the range of 3.0 - 6.0 μU/ml. It is accepted values above 10-20 μU/ml (60 pmol/L) be considered as a sign of insulin resistance (14,15,16). Here are the most commonly reported insulin values in OGTT: insulin less than 25 μU/ml or less than 174 pmol/L in the fasting state; 30-230 μU/ml or 208-1597 pmol/L at 30 minutes, 18-276 μU/ml or 125-1917 pmol/L at 60 minutes, 16-166 μU/ml or 111-1153 pmol/L at 120 minutes and less than 25 μU/ml or less than 174 pmol/L at 180 minutes. Fasting insulin levels in our controls ranged from 3.87 to 29.88 μU/ml, X_{mean} 13.40 ± 7,40. There was no registered zero concentrations of fasting insulin. The peak insulin value was achieved at 60 minutes, X_{mean} 93.72 ± 66.75, and then it decreased but remained higher than baseline. Compared with baseline, glucose levels increased 1.45 times at 60 minutes (Table 2) and 1.07 times at 120 minutes, and these of insulin 6.99 and 3.66 times, respectively. According to some authors, the increase of glucose ranges from 1.0 to 3.0 times and that of insulin from 4.5 to 11 times (2,6,20,21). It is assumed that insulin levels should never be 0 or less than 3 μU/ml. High concentrations in the fasting state are also problematic and indicative of IR or pre-diabetes, or early stage of DM2. The determination of fasting insulin serves also to differentiate DM2 from latent autoimmune diabetes in adults (LADA). In DM2, fasting insulin is normal or increased, while in LADA, it is typically decreased. The obtained results comply with the most data presented by other authors in recent years. The levels obtained during OGTT do not contradict those accepted by the ADA and the WHO. We believe that for our country, in view of the social and economic status, it is more reasonable to accept the WHO fasting glucose values, instead those suggested by the ADA.

Conclusions

Glucose and insulin levels in OGTT were monitored in 100 clinically healthy controls at 0, 60 and 120 minutes with the aim to establish our original reference ranges. Peak levels of both biomarkers were observed at 60 minutes after OGTT and these decreased at 120 minutes without reaching the baseline. Our results for glucose and insulin in OGTT are within those recommended by the WHO and the American Diabetes Association.

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