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PLASMA INSULIN RESPONSE TO ORAL GLUCOSE TOLERANCE TEST IN TYPE-2 NIGERIAN DIABETICS
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ABSTRACT

Objective: **To study the plasma insulin pattern in type 2 diabetic Nigerians both in the fasting state and in response to a standard oral glucose tolerance test.**
Design: **A cross sectional study.**
Setting: **Diabetic clinic, Ahmadu Bello University Teaching Hospital, Zaria Nigeria.**
Subjects: **Forty type 2 diabetic patients and thirty six healthy age and sex matched control subjects was undertaken.**
Interventions: **Eligible patients and control subjects underwent a standard oral glucose tolerance test (OGTT). Plasma levels of glucose and insulin levels determined by a glucose oxidase method and ELISA techniques respectively. Student's t-test was used to compare continuous variables, Chi-square test for categorical variable and Pearson's correlation coefficient to define correlation between variables. The level of statistical significance in each case was taken as $P < 0.05$.**
Results: **Type 2 diabetic patients demonstrated significantly lower fasting plasma insulin levels, when compared to control subjects (4.20 ± 1.78 micro- units/ml vs 5.72 ± 2.16 micro - units/ml respectively $p<0.05$). Similarly, plasma insulin levels following oral glucose challenge were significantly lower in the type 2 diabetic population.**
Conclusion: **Type 2 diabetic patients in this study demonstrate both fasting post OGTT hypoinsulinaemia. These findings are discussed in the light of the available knowledge on the aetiopathogenesis of type-2 diabetes mellitus in Africans.**

INTRODUCTION

Various abnormalities of insulin secretion have been described in patients with type 2 diabetes mellitus. In the fasting state, both hypoinsulinaemia and hyperinsulinaemia have been reported(1,2). Following glucose challenge, abnormalities such as an attenuation of the first phase response followed by an exaggerated second phase response, exaggeration of both phases and sometimes attenuation of both phases have been described(2,3). Racial factors seem to play modulatory roles in these diverse responses. In South Africa for example, Type 2 diabetic Africans exhibit lower plasma insulin levels compared to their Indian counterparts(4).

The few studies on plasma insulin levels in Nigeria concentrated on healthy volunteers and relatives of type 2 diabetic patients(5,6). There is as yet no reported study of the plasma insulin pattern in Nigerian type 2 diabetic patients as opposed to the vast literature on the subject in technically more developed countries. This study was designed to study the plasma insulin pattern in type 2 diabetic Nigerians both in the fasting state and in response to a standard OGTT. This could open room for more research for a better understanding of this heterogeneous metabolic disorder.

MATERIALS AND METHODS

All patients and control subjects studied were drawn from a single ethnic group (Hausa-Fulani) around the city of Zaria (located at Longitude 08° 30° East and latitude 04° 00° North) in Northern Nigeria. Type 2 diabetic patients attending the diabetic clinic of Ahmadu Bello University Teaching Hospital (ABU T H) Zaria and had 'good' glycaemic control, defined as fasting blood sugar (FBS) of 4.4 to 6.7 mmol/l, and or a 2 hour post prandial blood sugar of 4.4 to 8.9 mmol/l and 'accceptable' glycaemic control (FBS if 6.7 to 7.8 mmol/l and or 2 HPP of 8.9 to 10.0 mmol/l(7) on at least three clinic visits while on dietary therapy alone, or dietary therapy in addition to oral hypoglycaemic agent(s) formed the subjects of this study. Classification of patients as type 2 diabetic was however based on clinical grounds of non-dependence on insulin for survival(8). The exclusion criteria were insulin dependence, evidence of secondary diabetes, current insulin therapy, previous history of ketosis, pregnancy or use of oral contraceptives, and clinical or biochemical evidence of disease of the liver, kidney or thyroid. Alternate patients who met the inclusion criteria were selected.

Thirty six healthy, age, sex and socio economic status matched volunteers who had no personal or family history of diabetes mellitus or hypertension were recruited to serve as controls. The exclusion criteria were clinical evidence of any illness, personal or family history of diabetes mellitus or hypertension, current use of any form of medication and engagement in competitive sports.

Information on age, sex and anthropometric measures were obtained from all patients and control subjects. Weights (in Kilograms) were taken with only undergarments to the nearest 0.5 kg. Heights (in metres) were taken to the nearest 0.5 cm with subjects standing erect without shoes or headgear. Body Mass Index (BMI) was derived by dividing the weight by the square of the height(9).

Metabolic studies: Institutional ethical committee approval was granted before the study was done and informed consent was obtained from all patients and control subjects. All patients were instructed to stop oral hypoglycaemic agent therapy one week before metabolic studies to eliminate the effect of these drugs on insulin secretion(4).

Following an overnight 10-12 hours fast commencing between 21.00 to 22.00 hours the preceding night, 5ml of venous blood were drawn from each subject into EDTA treated tubes and promptly centrifuged. The plasma was then divided into aliquots for plasma glucose and insulin estimation. Glucose analysis was done within an hour of collection of the plasma using a glucose oxidize method(10). Aprotinin 200 KIU/ ml of plasma(11) was added to the aliquot for insulin assay; this was kept at - 20° Centigrade until analysis.

Following the withdrawal of the fasting sample, an 18G intravenous canular was left *in situ* with a slow infusion of saline (3-5 drops per minute) to maintain patency. Anhydrous glucose (75 grams dissolved in 300ml of water) was given to each subject, and each of them completed this within 5 minutes. The time of the first sip was recorded as time zero minute of the OGTT.

Blood samples were taken at times 30,60,90 and 120 minutes of the OGTT and handled the same way as the fasting blood samples. Patients and control subjects were observed in the metabolic laboratory and discharged home only when in satisfactory condition. Plasma insulin assays were performed using a commercially available ELISA human insulin kit (DRG instruments Gmbh, Mar burg, Germany, Cat no. EIA 2935). This kit has a sensitivity of 99% for human insulin, inter-assay and intra-assay coefficients of variation of 5.2% and 4.8% respectively and no cross-reaction with pro insulin.

Results are presented as mean ± standard deviation. Unpaired student's t-test was used to determine the differences between continuous variables and chi-square test was used for categorical variables. Pearson's correlation coefficient was used to define correlation between variables. The level of statistical significance in each case was taken as $P < 0.05$.

RESULTS

A total of 40 type 2 diabetic patients and 36 control subjects participated fully in the study. Table 1 summarises the age/sex distribution of the two groups of subjects. Average age at time of study was 49.4 ± 9.7 years (range 36 to 70 years) for type 2 diabetic patients and 48.6 ± 9.8 years (range 36 to 69 years) for control subjects ($P>0.5$). Similarly, the sex distribution for the two groups was also similar ($P > 0.5$). Nine (23%) of the control subjects were overweight compared to 16 (40%) of the type-2 diabetic patients ($p<0.05$). Type 2 diabetic patients had significantly higher body mass indices than control subjects (with respective mean and standard deviation values of 24.93 ± 4.43 Kg/M^2 for diabetic patients versus 22.93 ± 4.02 Kg/M^2 for controls ($p<0.05$).

Average duration of diagnosis of diabetes was 5.6 ± 4.3 years (range I to 20 years). Fifteen (37.5%), of the diabetic patients had 'good' glycaemic control, while 25 (62.5%) had 'acceptable' glycaemic control at entry into the study. All the diabetic patients required oral hypoglycaemic agents in addition to dietary measures for glycaemic control (25 on chlopropamide alone, 12 on chlopropamide and metformin and three on metformin alone). Blood sugar levels among diabetic and control subjects during the study and the levels of statistical significance of the differences are shown in Table 2.

Table 1

Age and sex distribution among type 2 diabetic patients and control subjects

Age range (years)	Diabetic patients				Control subjects				Total	
	Male		Female		Total		Male		Female			
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
30-39	5	(17.9)	3	(25)	8	(20)	4	(16.7)	3	(25)	7	(19.4)
40-49	8	(28.6)	5	(41.7)	13	(32.5)	10	(41.7)	4	(33.3)	14	(38.9)
50-59	10	(35.7)	3	(25)	13	(32.5)	5	(20.8)	3	(25)	8	(22.2)
≥60	15	(17.3)	1	(8.3)	6	(15)	5	(20.8)	2	(16.7)	7	(19.4)
Total	28	(70)	12	(30)	40	(100)	24	(66.7)	12	(33.3)	36	(100)

Due to rounding up some of the percentages do not add up to 100

Table 2

Plasma glucose levels among type 2 diabetic patients and control subjects

OGTT time (minutes)	Plasma glucose (mmol/L) Type 2 diabetic patients (n=40)	Control subjects (n=36)	{p-value (t-test)}
Fasting	9.77 ± 2.02	3.95 ± 0.49	<0.001
30	13.88 ± 2.57	7.55 ± 0.42	<0.001
60	17.5 ± 3.25	6.94 ± 0.54	<0.001
90	18.8 ± 3.27	6.45 ± 0.47	<0.001
120	17.98 ± 2.80	5.63 ± 0.51	<0.001

Values represent mean ± standard error of mean

Table 3

Plasma insulin levels among type 2 diabetic patients and control subjects

Plasma sample (minutes)	Type 2 diabetics (n=40) Plasma insulin (Mean ± SD)	Control subjects (n = 37) Plasma insulin (Mean ± SD)	p-value (t-test)
Fasting	4.20 ± 1.78 (Range 1.1 - 23.2)	5.72 ± 2.16 (Range 1.5 - 45)	<0.05
30	5.50 ± 1.84 (Range 1.4 - 27)	15.58 ± 2.51) (Range 4-105)	<0.001
60	6.01 ± 1.79 (Range 2.5 - 26.5)	13.67 ± 2.46 (Range 3.2 - 76.0)	<0.001
90	6.98 ± 1.91 (Range 4-24)	10.84 ± 2.41 (Range 2 - 61.0)	<0.05
120	9.92 ± 2.17 (Range 3.1 ± 23.5)	8.03 ± 2.38 (Range 2 - 61.0)	>0.1

Time in minutes is in relation to the OGTT. All values in micro-units/ml

There were marked variations in the individual fasting plasma insulin levels in both diabetic and control subjects, the same were observed following the OGTT. Table 3 summarises the mean ± SD, the range of values observed and levels of statistical significance of the differences between the two groups during OGTT, while Figure I is a scatter diagram of plasma insulin levels among type 2 diabetic patients and control subjects during fasting, 30 minutes and 120 minutes following oral glucose challenge. There was no significant difference between the fasting or post oral glucose challenge plasma insulin levels observed among type 2 diabetic patients who had 'good' or 'acceptable' control ($p>0.5$ in each case).

There was no significant correlation between plasma insulin levels and BMI among type 2 diabetic patients ($r = + 0.057$, $p>0.5$), similarly, the duration of diagnosis of diabetes did not correlate with fasting plasma insulin levels($r = -0.1333$, $p>0.5$).

DISCUSSION

Type 2 diabetic patients despite much higher blood glucose levels had relatively lower insulin output. This could be explained by the failure of the pancreatic beta cells in the diabetic patients to respond appropriately to the prevailing blood glucose levels. The fasting and post OGTT hypoinsulinaemia observed among type 2 diabetic patients in this study, is in agreement with earlier studies in Africa(4,12,13) and African-Americans(14), type 2 diabetic populations, but contrary to findings in most European studies where initial hypoinsulinaemia is demonstrated at 30 minutes of OGTT followed by delayed hyperinsulinaemia at 120 minutes of OGTT(1).

Following OGTT, the insulin response at 30 minutes representing the acute response was expectedly brisk in the control group. In the type 2 diabetic patients however, it was virtually non-existent similar to the

findings of previous investigators in Africa(4,12,13). Furthermore, the hyperinsulinaemia that was noted by Yalow and Berson(15) two hours following glucose ingestion was not demonstrated by type 2 diabetic patients in this study. This suggests that not only is there biochemical inertia in the initial insulin response by the beta-cells of type 2 diabetic patients in this study, but also a subnormal response in the later phase of insulin secretion. This pattern of relative hypoinsulinaemia at all times during the OGTT seems to be the rule in Africans, and African-American type 2 diabetic patients. In South Africa, Omar and Asmal(4) in a study of 14 young (aged less than 35 years) African patients with type 2 diabetes and 10 African controls demonstrated lower plasma insulin levels in the diabetic group in the fasting state as well as following the OGTT. This was similar to the findings of Asmal and Leary(12) in older African type 2 diabetic patients. Furthermore, Chaiken and colleagues(16) in a study of insulin sensitivity using the euglycaemic insulin clamp technique in 90 type 2 diabetic African-Americans (37 males and 53 females) found normal insulin sensitivity in 33% and 25% of the males and females respectively, suggesting that beta-cell malfunction is the underlying abnormality in a significant number of these patients.

It is indeed possible that the underlying defect in African type 2 diabetics is predominantly pancreatic beta cell malfunction. This could account for this state of fasting and post stimulatory hypoinsulinaemia. Osei *et al*(14), in a study of 154 African-Americans made up of 101 normoglycaemic controls, 36 individuals with impaired glucose tolerance (IGT), and 17 with mild type 2 diabetes. observed significant reduction in beta cell function in those with IGT and mild type 2 diabetes mellitus. The mild type 2 diabetics furthermore demonstrated lower serum insulin levels than the IGT group. This suggests that African-Americans even at near normal and normal blood glucose levels demonstrate some beta-cell dysfunction. This dysfunction is expected to get worse, as a result of hyperglycaemia if type 2 diabetes eventually develops as a result of glucotoxicity. This explanation may also be valid in the native Africans, as the genetic composition of African-Americans (the ancestors of whom are from Africa, are expected to a large extent to be similar to that of native Africans. However, in a comparative study of normoglycaemic African-Americans(18 first degree relatives of patients with type 2 diabetes and nine control subjects) and normoglycaemic Nigerians (20 first degree relatives of patients with type 2 diabetes and 18 healthy control subjects), Osei and colleagues(6) found significantly (three times) higher fasting serum insulin levels among African-American relatives of people with type 2 diabetes compared to their Nigerian counterparts. Furthermore, following intravenous glucose challenge, the African-Americans demonstrated two to three times greater acute-first and second phase insulin response than Nigerians irrespective of family history of diabetes. Their study suggests that the antecedent lesions leading to the development of type 2 diabetes among relatives of African-Americans type 2 diabetic patients may be different from their Nigerian counterparts, where hyperinsulinaemic response is not a feature even in their relatives.

Obesity is known to lead to insulin resistance with resultant hyperinsulinaemia(7-9). A positive correlation between plasma insulin levels and the degree of obesity is therefore expected and observed in most studies(19,20). In this study however, there was no significant correlation between plasma insulin levels and BMI. Similarly, Aronoff *et al.*(19) in a comparative study of plasma insulin level in Caucasians and Pima Indians had earlier on noted lack of association between degree of obesity and fasting plasma insulin levels among Caucasians but a positive association among Pima Indians. Furthermore, Chaiken and co-workers(16) in a study of 90 type 2 diabetic black patients, found no significant association (r-0.040, p= 0.82) between insulin sensitivity as determined by the euglycaemic clamp technique and BMI . These observations suggest that racial factors may be responsible for these observations.

We conclude that type 2 diabetic patients in this study demonstrate both fasting and post oral glucose challenge hypoinsulinaemia. However, because of the compounding effects of hyperglycaemia on both insulin secretion and insulin resistance, it is not possible to distinguish defects of insulin secretion or action that are pathogenically involved in the development of type-2 diabetes and those that result from the effects of hyperglycaemia(17,18). Larger prospective multi Center studies on insulin secretion and sensitivity are therefore needed to establish the relative roles of insulin secretion and sensitivity in Nigerian type 2 diabetic patients.

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