Diabetologia (1989) 32: 52-55

Diabetologia

Relationship between glucose tolerance, insulin secretion, and insulin action in non-obese individuals with varying degrees of glucose tolerance

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Summary. Plasma glucose and insulin concentration following a 75 g oral glucose challenge and glucose uptake during a hyperinsulinaemic glucose clamp study were determined in 50 non-obese individuals. The study population was divided into five groups on the basis of their glucose tolerance: normal, impaired glucose tolerance, Type 2 (non-insulin-dependent) diabetes mellitus with fasting plasma glucose of less than 8 mmol/l, between 8-15 mmol/l, and more than 15 mmol/l. The plasma insulin response was significantly greater ($p < 0.001$) than normal in those with either impaired glucose tolerance or Type 2 diabetes and a fasting plasma glucose concentration less than 8 mmol/l. In contrast, the plasma insulin response was similar to normal in the other two groups of patients with Type 2 diabetes, i.e. fasting plasma glucose concentration 8-15 mmol/l or greater than 15 mmol/l. Glucose uptake rates were significantly lower ($p < 0.001$) than normal in subjects with impaired glucose tolerance and all three groups of patients with Type 2 diabetes. Although glucose uptake rates during the glucose clamp studies were relatively similar in all four groups of glucose intolerant subjects, the values were significantly lower in those patients with Type 2 diabetes who had a fasting plasma glucose concentration greater than 8 mmol/l ($p < 0.01$). These data indicate that a significant degree of insulin resistance exists in patients with impaired glucose tolerance or Type 2 diabetes, relatively independent of fasting plasma glucose concentration. Indeed, glucose uptake during glucose clamp studies fell 8-fold over a range in fasting plasma glucose concentration of from 4.5 to 6.5 mmol/l. In contrast, the plasma insulin response increased over the same range of fasting plasma glucose concentrations. The fact that this defect in insulin action can be seen in patients who are hyperinsulinaemic, not hypoinsulinaemic, and only modestly hyperglycaemic, is consistent with the hypothesis that resistance to insulin-stimulate glucose uptake is a basic characteristic of patients with impaired glucose tolerance or Type 2 diabetes.

Key words: Type 2 diabetes, impaired glucose tolerance, insulin resistance, insulin response, glucose tolerance.

Although there seems to be general agreement [1, 2] that resistance to insulin-simulated glucose uptake exists in patients with Type 2 (non-insulin-dependent) diabetes mellitus, considerable questions remain as to the significance of this abnormality in the pathogenesis of Type 2 diabetes. In this context, a major issue is the relationship between insulin resistance and the other metabolic variables that are seen in patients with Type 2 diabetes. For example, it could be argued that insulin resistance is the basic defect in patients with Type 2 diabetes, and that hyperglycaemia ensues when the insulin secretory response is not sufficient to overcome the abnormality in glucose uptake. Alternatively, it has been proposed that a decrease in B-cell function is the primary abnormality in Type 2 diabetes, and that hyperglycaemia and insulin resistance occur secondarily [3]. It has also been suggested that resistance to insulin-stimulated glucose uptake and a reduced insulin secretory response are both the result of chronic hyperglycaemia [4]. One way to evaluate these different points of view is to define the relationship between the three variables involved, i.e. magnitude of hyperglycaemia, insulin action, and insulin secretion in individuals with varying degrees of glucose tolerance, and to select the formulation that most appropriately accounts for the experimental observations. The study to be described was initiated in an attempt to accomplish that task.

Materials and methods

Subjects

The study population consisted of 50 non-obese volunteers, classified as having normal glucose tolerance ($n = 10$), impaired glucose tolerance (IGT; $n = 10$), or Type 2 diabetes ($n = 30$), based on criteria recently published by the National Diabetes Data Group [5]. The pa-

Table 1. Clinical characteristics (Mean ± SEM)

Group	Non-diabetic subjects		Diabetic patients		
	Control	IGT	8 mmol/l	8–15 mmol/l	15 mmol/l
Age (years)	56.0 ± 4.0	59.0 ± 2.0	55.0 ± 4.0	57.0 ± 2.0	58.0 ± 2.0
BMI (kg/m^2)	27.9 ± 0.8	28.7 ± 1.1	27.5 ± 0.9	27.7 ± 1.3	27.9 ± 1.4
FPG (mmol/l)	5.1 ± 0.1	5.5 ± 0.1	6.6 ± 0.3	13.5 ± 0.6	19.4 ± 1.2

BMI = Body mass index; FPG = Fasting plasma glucose concentration; IGT = Impaired glucose tolerance

tients with Type 2 diabetes were further divided into three groups of 10 each on the basis of their fasting plasma glucose concentration: < 8 mmol/l, 8–15 mmol/l, or > 15 mmol/l. Volunteers were recruited into each of the categories until there were 10 subjects in each of the five groups, with a female to male distribution of approximately 50%. The patients with Type 2 diabetes had never been treated with insulin, nor had they received sulfonylurea compounds for at least one month before admission. All 50 subjects were in good general health, were physically active, had no other significant disease, and were not taking any drugs known to affect carbohydrate metabolism.

The degree of obesity was estimated by use of the body mass index (BMI = kg/m^2), based on the estimate that a BMI of 30 kg/m^2 was equivalent to being approximately 20% over ideal body weight [6]. All subjects had a BMI < 30 kg/m^2, and the degree of obesity was similar in all groups. These data, along with some other relevant clinical characteristics of the three groups, are shown in Table 1.

This study was approved by the Stanford University Human Subjects Committee, and each individual signed a consent form upon admission to the Stanford General Clinical Research Center. All were fed an isocaloric (35 Calories/kg) diet containing (as percentage of total calories) 17% protein, 40% fat, and 43% carbohydrate, and each meal had this relative content of nutrients. Meals, which were eaten at 08.00, 12.00, and 18.00 h, contained 20%, 40%, and 40%, respectively, of the day's total caloric intake. The experimental measurements to be described subsequently were initiated after at least 3 days of dietary stabilisation.

Experimental measurements

Oral glucose tolerance test. Blood was drawn for the measurement of fasting plasma glucose and insulin at 08.00 hours after an overnight fast. The subjects then were given a 75 g oral glucose load, and blood was drawn for measurement of plasma glucose [7] and insulin [8] concentrations 30, 60, 120, and 180 min later.

Glucose disposal rates. Measurements of glucose turnover rate were made during primed continuous infusions of (^{3}H)3-glucose as described previously [9]. These studies were initiated at 08.00 hours after an overnight fast and lasted for 4 h. The subjects were maintained at their basal plasma glucose throughout the study, while steady state plasma insulin concentrations were raised to approximately 100 mU/l. Arterialised venous blood samples were obtained from an in-dwelling catheter inserted retrograde into a hand vein placed in a radiant warmer maintained at 70° C. Plasma was immediately separated in a Beckman microfuge (model S, Beckman, Fullerton, Calif, USA), and glucose was determined with a Beckman Glucose Analyzer II. After determining the baseline plasma glucose concentration, a primed continuous infusion of insulin (40 mU/m^2 · min^{-1}) was started. Plasma glucose was determined every 5 min thereafter. A variable infusion of glucose was started 4 min after the start of the insulin infusion and was adjusted to maintain plasma glucose within 10% of the baseline value using a negative feedback algorithm.

The rate of disappearance of glucose (total glucose disposal (Rd)) was calculated during the last hour of the clamp period. To do this, aliquots of plasma were collected at 30-min intervals, precipitated with $Ba(OH)_2$ and $ZnSO_4$, centrifuged, and the protein-free supernatant evaporated in a scintillation vial. Plasma glucose concentrations and radioactivity were measured, glucose specific activity determined, and Rd calculated using the nonsteady state equation of Steele [10]. To quantify tissue uptake of glucose, it is necessary to subtract the rate of urinary glucose loss from the Rd. Finally, since Rd varies as a function of the plasma glucose concentration [11], the glucose metabolic clearance rat (MCR) was calculated by dividing the tissue uptake of glucose (Rd – urinary glucose) by the plasma glucose concentration determined during the clamp study.

Statistical analysis

Data are expressed as the mean ± SEM and were analysed by the Statistical Analysis System using the general linear models procedure. To evaluate the differences in plasma glucose and insulin response to oral glucose in patients with abnormal carbohydrate metabolism and normal subjects, data were analysed [12, 13] by two-way analysis of variance (ANOVA). Scheffe's multiple comparison test was employed to evaluate the differences between any two groups of the five groups we studied.

Results

Mean (± SEM) plasma glucose responses of the five experimental groups are shown in Figure 1. It is apparent from these data that the plasma glucose response of the five groups increased progressively from those with normal glucose tolerance to patients with Type 2 diabetes and a fasting plasma glucose concentration in excess of 15 mmol/l. When compared by Scheffe's multiple comparison test, it was found that the glucose response of any given group was different from that of the other four groups ($p < 0.001$).

Figure 2 displays the mean (± SEM) plasma insulin responses of the same five groups, and it is obvious that the relationship between degree of glucose intolerance, and the insulin response was quite complicated and difficult to describe simply. When compared by Scheffe's multiple comparison test, the insulin response of patients with impaired glucose tolerance was significantly higher ($p < 0.05$) than the other four groups. Patients with Type 2 diabetes and the lowest mean fasting plasma glucose concentration (< 8 mmol/l) also had an insulin response which was significantly ($p < 0.05$) greater than that of the normal subjects. On the other hand, the plasma insulin response of the two other groups of patients with Type 2 diabetes was not significantly lower than that of the group with normal oral glucose tolerance.

Mean (± SEM) glucose disposal rates during the glucose clamp study are seen in Figure 3. It can be seen that the values were dramatically lower than normal in all four patient groups ($p < 0.001$). However, there was

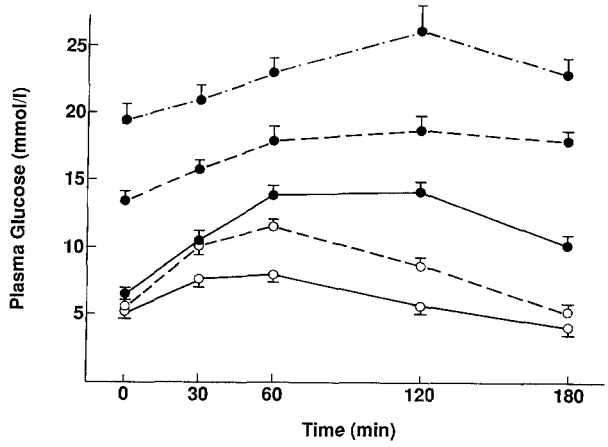

Fig. 1. Mean (±SEM) plasma glucose concentrations in the five study groups before and 30, 60, 120, and 180 min after a 75 g oral glucose challenge. Type 2 (non-insulin-dependent) diabetes (●—●; <8 mmol/l), (● — ●; 8-15 mmol/l), (●----●; >15 mmol/l), normal (○—○), impaired glucose tolerance (○----○)

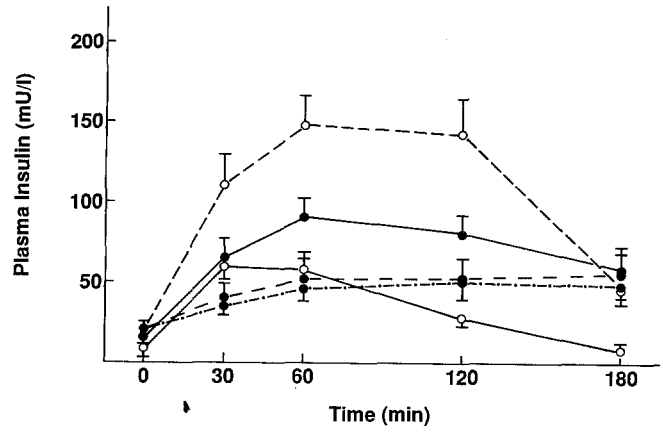

Fig. 2. Mean (±SEM) plasma insulin concentrations in the five study groups before and 30, 60, 120, and 180 min after a 75 g oral glucose challenge. Type 2 (non-insulin-dependent) diabetes (●—●; <8 mmol/l), ● — ●; 8-15 mmol/l), (●----●; >15 mmol/l), normal (○—○), impaired glucose tolerance (○----○). □ Non-diabetic subjects; ■ Type 2 (non-insulin-dependent) diabetic patients

no significant difference between the glucose clearance values in patients with either impaired glucose tolerance or Type 2 diabetes and a fasting plasma glucose concentration <8 mmol/l. Glucose uptake rates were also similar between the two groups of patients with Type 2 diabetes and fasting glucose concentration >8 mmol/l, but values were lower in both of these groups when compared to those with impaired glucose tolerance or Type 2 diabetes and fasting glucose concentration <8 mmol/l ($p<0.01$). Mean (±SEM) plasma insulin concentration during the clamp studies were similar in the five groups - 95±4, 95±3, 94±3, 97±4, and 96±4 mU/l, respectively.

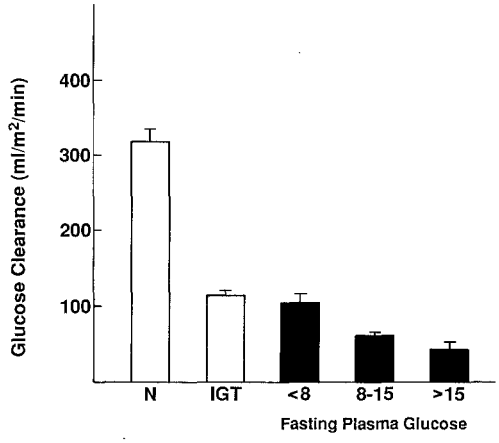

Fig. 3. Mean (±SEM) glucose metabolic clearance rates rates calculated from the results of glucose clamp studies in the five study groups. *N*=Normal glucose tolerance, IGT=Impaired glucose tolerance, <8=Fasting glucose (<8 mmol/l), 8-15=Fasting glucose (8-15 mmol/l), >15=Fasting glucose (>15 mmol/l). □ Non-diabetic subjects; ■ Type 2 (non-insulin-dependent) diabetic patients

Discussion

To the best of our knowledge, this report represents the only study in which the relationship between glucose tolerance, plasma insulin response to glucose, and insulin-simulated glucose uptake has been defined in a large number of non-obese individuals over a wide range of fasting plasma glucose concentration. As such, the results are quite similar to those of previous studies carried out in a smaller number of obese individuals - in one case Caucasian [14] and the other, Pima Indians [15]. In all three studies, it is apparent that very small increases in degree of glycaemia are associated with precipitous declines in insulin-stimulated glucose uptake, with an approximate 8-fold fall in glucose uptake as fasting plasma glucose concentration goes from 4 to 8 mmol/l. In contrast, values for insulin-stimulated glucose uptake are quite similar in patients with Type 2 diabetes over a range of plasma glucose concentration from approximately 8 to 20 mmol/l. In some respects, the variations in plasma insulin response to glucose as a function of degree of glycaemia are similar to those seen in insulin action, with relatively dramatic changes in insulin response seen over a fasting plasma glucose concentration range of from 4-8 mmol/l, and relatively little difference in insulin response when fasting plasma glucose concentration exceeds 8 mmol/l. However, the relationship between these variables in the various diagnostic categories is quite different. Thus, the plasma insulin response to glucose is higher than normal in patients with impaired glucose tolerance or Type 2 diabetes and fasting plasma glucose concentration <8 mmol/l, whereas their values for glucose uptake are lower than normal. When considering patients with Type 2 diabetes and a fasting plasma glucose concentration >8 mmol/l, it was seen that their plasma insulin response to glucose was not significantly different from that of individuals with normal glucose tolerance, but their glucose uptake values during the clamp studies were certainly lower than normal. Any formulation of

the relationship between defects in insulin action and secretion must be based upon this data base.

It is clear from the data that plasma insulin concentration was greater than normal in patients with either impaired glucose tolerance or Type 2 diabetes and a fasting plasma glucose concentration < 8 mmol/l. Since glucose uptake during the glucose clamp studies in these two groups was only one-third the value of the normal control subjects, it is obvious that insulin resistance in these two groups is not secondary to absolute hypoinsulinaemia. Indeed, plasma insulin responses in patients with Type 2 diabetes and fasting glucose concentration > 8 mmol/l were not significantly lower than the normal response in absolute terms. This is not meant to imply that B-cell function is normal in patients with Type 2 diabetes, as it could be argued that hyperglycaemic individuals should have higher than normal plasma insulin concentrations. However, these data are consistent with results of other studies in human beings [1, 2, 14–16] which indicate that insulin resistance can exist in Type 2 diabetes in the absence of hypoinsulinaemia. Furthermore, it is difficult to account for the current findings on the basis of "glucotoxicity" [4]. As emphasised earlier, the major decline in glucose uptake took place over an extremely narrow glycaemic range, i.e. from a fasting glucose concentration of from 4.0 to 7.0 mmol/l. If this decline was primarily due to glucotoxicity, it must be argued that glucose uptake is extremely sensitive to small changes in plasma glucose concentration within the normal range, and quite resistant to the deleterious effect of increases in plasma glucose concentration once unequivocal fasting hyperglycaemia has been achieved. Since plasma insulin levels are higher than normal, not lower, in patients with impaired glucose tolerance or mild Type 2 diabetes, it is necessary to propose that glucotoxicity in these particular patients reduces insulin action but not insulin secretion.

The view that insulin resistance can develop in the absence of either absolute hypoinsulinaemia or glucotoxicity does not mean that these variables do not modulate insulin action in patients with Type 2 diabetes. Nor can these cross-sectional data serve as evidence for the sequence of events that occur in this syndrome. On the other hand, they can serve as the framework for a hypothesis as to the etiology of Type 2 diabetes. Specifically, we would speculate that resistance to insulin-stimulated glucose uptake is the basic defect in patients with glucose intolerance. If such individuals are capable of secreting large amounts of insulin, they can prevent frank decompensation of glucose homostasis. When that hyperinsulinaemic state cannot be maintained, for whatever reason, fasting hyperglycaemia develops. We believe that this formulation fits the results that have been presented, and that no other hypothesis explains these observations as successfully. Moerover, it is a hypothesis that can be tested experimentally in a longitudinal study. Whether or not it is found ultimately to be true, remains to be seen.

Acknowledgements. Supported by Research Grants from the Nora Eccles Treadwell Foundation and the National Institutes of Health (DK-30732 and RR-7027).

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Received: 11 July 1988X
and in revised form: 7 November 1988

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