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Abstract

Background. The relative roles of obesity, insulin resistance, insulin secretory dysfunction, and excess hepatic glucose production in the development of non-insulin-dependent diabetes mellitus (NIDDM) are controversial. We conducted a prospective study to determine which of these factors predicted the development of the disease in a group of Pima Indians.

Methods. A body-composition assessment, oral and intravenous glucose- tolerance tests, and a hyperinsulinemic-euglycemic clamp study were performed in 200 non-diabetic Pima Indians (87 women and 113 men; mean [+SD] age, 26+-6 years). The subjects were followed yearly thereafter for an average of 5.3 years.

Results. Diabetes developed in 38 subjects during follow-up. Obesity, insulin resistance (independent of obesity), and low acute plasma insulin response to intravenous glucose (with the degree of obesity and insulin resistance taken into account) were predictors of NIDDM. The six- year cumulative incidence of NIDDM was 39 percent in persons with values below the median for both insulin action and acute insulin response, 27 percent in those with values below the median for insulin action but above that for acute insulin response, 13 percent in those with values above the median for insulin action and below that for acute insulin response, and 0 in those with values originally above the median for both characteristics.

Conclusions. Insulin resistance is a major risk factor for the development of NIDDM. A low acute insulin response to glucose is an additional but weaker risk factor.

Keywords

precursors, secretory, resistance, indians, pima, insulin, studies, dysfunction, prospective, mellitus, diabetes, dependent, non

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INSULIN RESISTANCE AND INSULIN SECRETORY DYSFUNCTION AS PRECURSORS OF NON-INSULIN-DEPENDENT DIABETES MELLITUS

Prospective Studies of Pima Indians

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Abstract Background. The relative roles of obesity, insulin resistance, insulin secretory dysfunction, and excess hepatic glucose production in the development of non-insulin-dependent diabetes mellitus (NIDDM) are controversial. We conducted a prospective study to determine which of these factors predicted the development of the disease in a group of Pima Indians.

Methods. A body-composition assessment, oral and intravenous glucose-tolerance tests, and a hyperinsulinemic-euglycemic clamp study were performed in 200 nondiabetic Pima Indians (87 women and 113 men; mean [$\pm$ SD] age, 26 ± 6 years). The subjects were followed yearly thereafter for an average of 5.3 years.

Results. Diabetes developed in 38 subjects during follow-up. Obesity, insulin resistance (independent of obesity),

and low acute plasma insulin response to intravenous glucose (with the degree of obesity and insulin resistance taken into account) were predictors of NIDDM. The six-year cumulative incidence of NIDDM was 39 percent in persons with values below the median for both insulin action and acute insulin response, 27 percent in those with values below the median for insulin action but above that for acute insulin response, 13 percent in those with values above the median for insulin action and below that for acute insulin response, and 0 in those with values originally above the median for both characteristics.

Conclusions. Insulin resistance is a major risk factor for the development of NIDDM. A low acute insulin response to glucose is an additional but weaker risk factor. (N Engl J Med 1993;329:1988-92.)

THE most common form of non-insulin-dependent diabetes mellitus (NIDDM) is characterized by obesity, insulin resistance, insulin secretory dysfunction, and overproduction of glucose in the liver. The relative roles of these metabolic abnormalities in the causation of NIDDM remain controversial,^{1,2} because once the disease has developed it is impossible to determine the initial events. Cross-sectional studies of subjects at high risk for NIDDM provide some information about the characteristics that may lead to the development of the disease, but these studies are limited by the lack of knowledge of which subjects will indeed go on to have the disease. Only prospective studies can determine the risk factors underlying the pathogenesis of NIDDM.

Such studies³⁻¹³ have provided some insight into this question, but the extent of the physiologic assessment has been limited. In this study of nondiabetic Pima Indians, we measured body composition, ability to secrete insulin, and insulin action in vivo, using the hyperinsulinemic-euglycemic clamp technique to obtain comprehensive data about insulin secretion and action. We then followed the subjects annually to detect the development of NIDDM and compared the results in those in whom the disease developed and those in whom it did not.

METHODS

Study Subjects

From 1982 through 1992, we studied 200 healthy, nondiabetic Pima Indians, including 87 women and 113 men, with a mean

($\pm$ SD) age of 26 ± 6 years. The subjects were asked to return each year for testing that included an oral glucose-tolerance test to detect the presence of diabetes, as defined by the World Health Organization.¹⁴ (Data on many of these subjects have appeared previously.¹⁴) The study protocol was approved by the ethics committees of the National Institutes of Health and the Indian Health Service, as well as by the Gila River Indian Community. The subjects gave informed consent.

Base-Line Assessment

The subjects were admitted to the clinical research unit for 8 to 15 days, during which they followed a weight-maintaining diet. The waist circumference of each subject was measured at the umbilicus, and the thigh circumference at the gluteal fold. The percentages of body fat and fat-free body mass were determined by underwater weighing.^{17,18} A 75-g oral glucose-tolerance test was performed, and the glucose-tolerance status of each patient was categorized according to the criteria of the World Health Organization.¹⁴ At this base-line test, glucose tolerance was normal in 151 patients and impaired in 49. The acute plasma insulin response to glucose was determined on the basis of an intravenous glucose-tolerance test in which 25 g of dextrose was injected intravenously for 3.6 minutes¹⁹ and blood samples were collected with the patient fasting and at 3, 4, and 5 minutes. The acute insulin response was defined as the incremental area under the curve from the third to the fifth minute after the dextrose injection, divided by two. A two-step study using a hyperinsulinemic-euglycemic clamp (at approximately 100 mg of glucose per deciliter [$5.6\text{ mmol per liter}$]) was performed to measure the action of insulin, as previously described.¹⁵ The mean ($\pm$ SE) steady-state low and high plasma insulin concentrations achieved were $130\pm 3\text{ }\mu\text{U per milliliter}$ ($780\pm 18\text{ pmol per liter}$) and $2072\pm 37\text{ }\mu\text{U per milliliter}$ ($12,432\pm 222\text{ pmol per liter}$), respectively. Before and during the low-dose insulin infusion, tracer amounts of [$3\text{-}^3\text{H}$]glucose were infused to permit the calculation of the rate of glucose disappearance.²⁰ The effects of variations in plasma glucose concentrations during the clamp study were adjusted to 100 mg per deciliter, as suggested by Best et al.²¹ Differences between individual subjects in insulin concentrations during the low-dose insulin infusion were taken into account in the calculation of the rate of glucose uptake, as previously described.^{15,22} Glucose uptake rates were normalized to metabolic body size, calculated as the fat-free body mass plus 14 kg, since metabolic rate is not directly proportional to fat-free body mass.²³ Suppression of basal endogenous glucose production was determined by calculating the difference between the rate of glucose appearance and the exogenous

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glucose infusion, subtracted from the rate of basal endogenous glucose production, and dividing the difference by the basal rate of endogenous glucose production, with the final value expressed as a percentage.

Intravenous glucose-tolerance tests were performed at base line in only 104 of the 200 subjects, but they were done later in 37 of the remaining subjects. Thus, the data on acute insulin responses were obtained from 141 rather than 200 subjects. Of these 141 subjects, 3 who had intravenous glucose-tolerance tests did not subsequently have euglycemic-clamp studies, leaving a total of 138 subjects in the analysis.

Statistical Analysis

Risk factors for NIDDM were estimated by proportional-hazards analysis.²⁴ The effects of continuous variables were expressed as relative hazards derived from these models and were evaluated at the 90th and 10th percentiles of the predictor variables. For a factor positively associated with NIDDM, the relative hazard estimates the hazard for a hypothetical subject at the 90th percentile divided by the hazard for a subject at the 10th percentile (or for the 10th and 90th percentiles, in the case of a negatively related variable). The analyses were adjusted for sex and sometimes for other variables. Ninety-five percent confidence limits are given for each relative hazard. Risk factors were also assessed by stratification. Within groups defined as having values above or below the median for insulin action or acute insulin response, the six-year cumulative incidence of NIDDM was estimated by the Kaplan-Meier method,²⁴ which makes no assumptions about the distribution of survival times.

RESULTS

Among the 87 women and 113 men who were followed for a mean of 5.3 years (range, 0.5 to 8.9), NIDDM developed in 38 subjects (24 women and 14 men) after a mean follow-up of 3.9 years.

Body Size and Plasma Glucose and Insulin Concentrations

Proportional-hazards analysis indicated that NIDDM was more likely to develop in the most obese subjects (Table 1). The ratio of waist to thigh circumference, an estimate of the central distribution of body fat, was also a strong predictor of NIDDM. The percentage of body fat was not a predictor after adjustment for sex and the ratio of waist to thigh circumference, but after adjustment for sex and percentage of body fat, the ratio of waist to thigh circumference continued to be a predictor of NIDDM (relative hazard, 9.1; 95 percent confidence interval, 2.5 to 33.4). Higher fasting plasma glucose and insulin concentrations and higher concentrations 30 minutes and 120 minutes after oral glucose administration were all predictors of NIDDM (Table 1).

Insulin Resistance and Hepatic Glucose Production

Glucose uptake at mean ($\pm$ SE) plasma insulin concentrations of 130 ± 3 μ U per milliliter (M_{130}) during the euglycemic-clamp study was the strongest single predictor of NIDDM (Table 1). The cumulative six-year incidence of NIDDM was 25 percent in persons with an M_{130} below the median, as compared with 9 percent in those with values above the median. M_{130} remained a strong predictor after adjustment for percentage of body fat (relative hazard, 21.2; 95 percent confidence interval, 3.2 to 141.4) and for the percentage of body fat and the ratio of waist to thigh cir-

Table 1. Risk Factors for the Development of NIDDM in 200 Pima Indians.

FACTOR*	VALUES AT 10TH OR 90TH PERCENTILE†	RELATIVE HAZARD‡	95% CONFIDENCE INTERVAL§
Body fat (%)	22, 52	7.8	2.3–26.8
Ratio, waist to thigh circumference	1.4, 1.8	12.2	4.0–36.8
Plasma glucose (mg/dl)¶			
Fasting	81, 101	2.4	1.0–5.4
30 min	114, 180	4.6	2.0–4.6
120 min	90, 161	8.6	3.7–20.0
Plasma insulin (μ U/ml)¶			
Fasting	16, 65	15.8	5.4–46.7
30 min	112, 457	4.7	1.9–11.6
120 min	51, 384	14.0	4.8–40.6
M_{130} (mg glucose/kg MBS · min)	4.4, 2.0	31.1	4.9–197.1
M_{2072} (mg glucose/kg MBS · min)	12.5, 6.8	5.0	2.2–11.4
Hepatic glucose production (mg/kg of MBS · min)			
Basal	1.79, 2.47	1.3	0.3–1.9
Percent suppression during M_{130}	100, 53	3.2	1.5–7.0
Acute insulin response (μ U/ml)	402, 104	2.2	0.9–5.9

* M_{130} denotes the glucose uptake rate at a mean ($\pm$ SE) plasma insulin concentration of 130 ± 3 μ U per milliliter during euglycemia, MBS metabolic body size (see the Methods section), and M_{2072} the glucose uptake rate at a mean plasma insulin concentration of 2072 ± 37 μ U per milliliter during euglycemia. To convert values for plasma glucose to millimoles per liter, multiply by 0.056; to convert values for plasma insulin to picomoles per liter, multiply by 6; to convert values for glucose uptake to micromoles per kilogram of MBS per minute, multiply by 5.6.

†The first value in this column is the value associated with a lower risk of NIDDM.

‡Relative hazards, computed by proportional-hazards analysis, represent the hazard rate for a subject at the 90th percentile divided by the rate for a subject at the 10th percentile (or the reverse, in the case of negatively related variables). The results for each variable were adjusted for sex, but not for other variables.

§A lower confidence limit greater than 1.0 indicates a relative hazard significantly greater than 1.0 ($P < 0.05$).

¶Values at 30 and 120 minutes were obtained during the oral glucose-tolerance test.

||As estimated from measurements with a glucose tracer.

cumference (relative hazard, 14.6; 95 percent confidence interval, 2.1 to 98.8). If the percentage of body fat, the ratio of waist to thigh circumference, and M_{130} were all included in the model, the percentage of body fat was not a predictor of NIDDM, whereas the ratio of waist to thigh circumference was (relative hazard, 6.0; 95 percent confidence interval, 1.6 to 21.7).

Low glucose uptake at high plasma insulin concentrations (2072 ± 37 μ U per milliliter) (M_{2072}) during the euglycemic-clamp study was also a predictor of NIDDM (Table 1). Like M_{130} , M_{2072} was associated with an increased risk of NIDDM even after adjustment for the percentage of body fat (relative hazard, 4.2; 95 percent confidence interval, 1.8 to 9.9) or for the percentage of body fat and the ratio of waist to thigh circumference (relative hazard, 4.2; 95 percent confidence interval, 1.5 to 11.6).

The rate of hepatic glucose production in the post-absorptive (basal) state was not predictive of NIDDM (Table 1). However, the suppression of hepatic glucose production at a plasma insulin concentration of approximately 130 μ U per milliliter during the euglycemic-clamp study was predictive (Table 1). After adjustment for the percentage of body fat and the ratio of waist to thigh circumference, the suppression of hepatic glucose production was not a signif-

icant predictor of NIDDM (relative hazard, 2.2; 95 percent confidence interval, 0.9 to 5.0).

Acute Plasma Insulin Response

Among the 141 subjects (61 women and 80 men) who had intravenous glucose-tolerance tests, NIDDM developed in 27 (16 women and 11 men) after a mean follow-up of 4.6 years. The acute plasma insulin response alone was not a significant predictor of the development of NIDDM (Table 1). However, the response was predictive after adjustment for percentage of body fat (relative hazard, 2.9; 95 percent confidence interval, 1.2 to 7.5) or for the percentage of body fat and the ratio of waist to thigh circumference (relative hazard, 2.7; 95 percent confidence interval, 1.0 to 7.1).

Relative Effects of M_{130} and the Acute Insulin Response

The relative effects of M_{130} and the acute insulin response on the risk of NIDDM are shown in Figures 1 and 2. The six-year cumulative incidence of NIDDM was 39 percent in persons with values below the median for both M_{130} and acute insulin response, 27 percent in those with values below the median for M_{130} but above the median for acute insulin response, 13 percent in those with values above the median for M_{130} and below the median for acute insulin response, and 0 in those with values above the median for both M_{130} and acute insulin response (Fig. 2).

In a proportional-hazards analysis using a model that included M_{130} , acute insulin response, and sex, M_{130} was a strong predictor of NIDDM (relative hazard, 52.7; 95 percent confidence interval, 5.5 to 506.1),

Figure 1. Acute Insulin Response and Glucose Uptake at Plasma Insulin Concentrations of 130 ± 3 μ U per Milliliter at Base Line in Pima Indians in Whom NIDDM Did ($\blacktriangle$) or Did Not ($\square$) Develop. For comparison, the mean value for 14 young, lean, nondiabetic white persons of normal weight is shown by a cross. Acute insulin response was measured for two minutes during an intravenous glucose-tolerance test, and glucose uptake in milligrams per minute per kilogram of metabolic body size (see the Methods section). To convert values for glucose uptake to micromoles per kilogram of metabolic body size per minute, multiply by 5.6. To convert values for plasma insulin to picomoles per liter, multiply by 6.

Figure 2. Six-Year Cumulative Incidence of NIDDM in Persons with Values Lower or Higher Than the Median for Glucose Uptake during Hyperinsulinemia (130 ± 3 μ U per Milliliter) and for Acute Insulin Response.

and acute insulin response was a weak predictor (relative hazard, 3.2; 95 percent confidence interval, 1.2 to 8.8). When the percentage of body fat and the ratio of waist to thigh circumference were included in the model, M_{130} remained a much stronger predictor (relative hazard, 30.8; 95 percent confidence interval, 2.8 to 34.4) than acute insulin response (relative hazard, 2.8; 95 percent confidence interval, 1.0 to 8.3). In this model, neither the percentage of body fat nor the ratio of waist to thigh circumference was a significant predictor of NIDDM.

Subjects with Normal Glucose Tolerance

Among the 151 subjects with normal glucose tolerance at base line, NIDDM developed in 17, and the risk factors were similar (data not shown). Insulin resistance was the strongest predictor of NIDDM, and a low acute insulin response was predictive only after adjustment for insulin resistance.

DISCUSSION

Insulin resistance was the strongest predictor of NIDDM in the group of Pima Indians we studied. This result agrees with inferences from more limited studies. Warram et al.⁹ and subsequently Martin et al.²⁵ reported that on the basis of the results of intravenous glucose-tolerance tests among white subjects, insulin resistance predicted NIDDM in the offspring of parents with NIDDM. Hyperinsulinemia, an indirect measure of insulin resistance, also predicts NIDDM in the Pimas,⁸ in Swedish women,¹² French police officers,¹³ and Mexican Americans.¹⁰

The degree of obesity, as estimated from measures of height and weight, is also a well-recognized predic-

tor of NIDDM,^{4,5,7-10,13} but because obesity and insulin resistance are often associated, the predictive effect of obesity may be due to insulin resistance. The studies in whites⁹ and Mexican Americans¹⁰ suggested that insulin resistance, estimated from an intravenous glucose-tolerance test or inferred from hyperinsulinemia, may be a stronger predictor of NIDDM than obesity. The degree of obesity was not measured directly in these previous studies, however. In the present study, insulin resistance and body composition were measured directly, and the degree of obesity had little or no effect in predicting NIDDM when insulin resistance was taken into account. Central obesity, which predicts NIDDM in other populations,²⁶ was also predictive in Pima Indians and remained a significant risk factor when percentage of body fat and insulin resistance were taken into account, but not when the acute insulin response was also considered. On the other hand, the predictive effect of insulin resistance remained strong when obesity, an estimate of central obesity, and the acute insulin response were taken into account. Although the overall effect of obesity may have been underestimated because the majority of our subjects were obese, insulin resistance was a predictor of NIDDM as a result of factors other than obesity alone. Because insulin resistance measured by the hyperinsulinemic-euglycemic clamp technique largely results from decreased rates of glycogen synthesis in skeletal muscle,²⁷ insulin resistance in skeletal muscle is predictive of NIDDM.

Hepatic overproduction of glucose did not predict NIDDM and was therefore a secondary abnormality occurring in the natural history of the disease. Decreased suppression of the rate of hepatic glucose production during the insulin infusion was a predictor of NIDDM, but this was largely accounted for by obesity; after adjustment for obesity and central obesity, suppression of hepatic glucose production was not a significant predictor of NIDDM.

The acute insulin secretory response to glucose, considered as a single variable, did not predict NIDDM, a result consistent with the findings of Warram et al.⁹ in the offspring of white diabetic parents. Only when the acute insulin response was considered together with the degree of obesity or insulin action did it significantly predict NIDDM. Similarly, Lundgren et al.¹² reported a weak predictive effect for a low acute insulin response, which strengthened when the fasting plasma insulin concentration, an estimate of insulin resistance, was taken into account.

Although insulin resistance and a low insulin response to glucose were predictive of NIDDM, the sequence of events in the evolution from normal glucose tolerance to fasting hyperglycemia is unknown. From cross-sectional and sequential studies,^{1,2,14} it appears that insulin resistance worsens as a result of increasing obesity, aging, or other unknown factors and that glucose tolerance worsens concomitantly. In response to increasing glycemia, insulin secretion increases, limiting increases in plasma glucose concentrations. Even-

tually, the insulin secretory response declines, and hepatic glucose production and plasma glucose concentrations increase in parallel with the decline in plasma insulin concentrations. The causes of this decline in insulin secretory response are unknown, but they may include the effects of aging or prolonged, mild hyperglycemia, so-called glucose toxicity.²⁸ Detailed knowledge of the pathophysiologic mechanisms of the loss of insulin secretory function and the increase in hepatic glucose production will be needed to understand how the primary etiologic factors, insulin resistance and low acute insulin responses, lead to the development of NIDDM.

Finally, are the results of this study in Pima Indians relevant to other persons with NIDDM? The Pimas are of Asian origin and thus represent the most numerous racial group on earth. NIDDM is phenotypically the same in the Pimas as in many whites, Mexican Americans, and blacks,^{1,2,9,29} suggesting a similar causation. There are exceptions, however. Whites in whom NIDDM develops at a young age (maturity-onset diabetes of the young) are not obese or insulin-resistant when the disease develops,³⁰ and some have mutations in the glucokinase gene that alter insulin secretory function.³¹ Also, some blacks with NIDDM are not insulin-resistant,²⁹ and a small proportion of subjects with NIDDM are lean and apparently have a slow onset of insulin-dependent diabetes mellitus.³² The majority of persons with NIDDM throughout the world, however, have metabolic characteristics similar to those of Pima Indians with NIDDM. We conclude, therefore, that the etiologic factors that result in NIDDM in Pimas are probably similar to those in other racial groups but that the genes that determine susceptibility to the disease are more common or more penetrant in the Pimas.

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