In Vivo β-Cell Function at the Transition to Early Non–Insulin-Dependent Diabetes Mellitus

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Impaired insulin secretion occurs at some stage in the development of non–insulin-dependent diabetes mellitus (NIDDM), possibly during impaired glucose tolerance (IGT) or early NIDDM. To assess insulin secretion at these critical stages, we measured the first-phase insulin response (to glucose and arginine), maximal secretory capacity, and glucose potentiation slope for insulin secretion in Pima Indians with normal glucose tolerance (n = 20), IGT (n = 9), and mild (fasting glucose < 7.8 mmol/L) NIDDM (n = 7). We also measured oral glucose tolerance and insulin action. Subjects with IGT were more insulin-resistant ($P < .05$) than normals. A wide range of insulin secretion was noted, although as a group, no significant impairment was detected. Subjects with mild NIDDM were similarly insulin-resistant, but they also had impaired insulin secretion. The first-phase response to glucose was markedly reduced in absolute terms ($P < .001$), but all secretion indices were impaired relative to the degree of insulin resistance ($P = .05$ to $P < .0001$). These results suggest that in Pima Indians, impairment of insulin secretion, especially the first-phase response to glucose, is associated with mild NIDDM. Insulin secretion in IGT is variable and, overall, seems intact, although a subtle defect in the first-phase insulin response to glucose could not be ruled out in this study. Glucose sensing for first-phase secretion may be one of the early secretory defects in the progression of glucose intolerance and seems to be critical at the transition from IGT to early NIDDM.

THE PIMA INDIANS of Arizona have the highest reported prevalence of non–insulin-dependent diabetes mellitus (NIDDM) in the world and, as a relatively genetically homogeneous group, provide an opportunity to study obesity-related NIDDM, the most common form of diabetes worldwide.[1]

NIDDM with fasting hyperglycemia greater than 7.8 mmol/L is characterized by skeletal muscle insulin resistance, impaired first- and second-phase insulin secretion, and unsuppressed hepatic glucose production.[2] The sequential appearance of these defects in the development of NIDDM may be determined by examining subjects with more intermediate degrees of glucose intolerance, and such studies may help determine the pathogenesis of the disease. Current evidence suggests that insulin resistance occurs relatively early in the evolution to NIDDM.[3-5] Elevated basal hepatic glucose production appears to be a late phenomenon, explaining to a large degree the increases in fasting glucose above 7.8 mmol/L.[2,6]

An abrupt change in plasma insulin concentrations relative to glucose concentrations is apparent from cross-sectional and longitudinal studies, appearing to coincide with the earliest sign of NIDDM.[3,4] This downturn in insulin concentration is widely thought to reflect β-cell failure, the decompensation limb of Starling's curve of the pancreas,[2] but is there evidence of secretory impairment before the onset of overt diabetes? Some studies in white[7-9] and Japanese[10] subjects with impaired glucose tolerance (IGT) have shown an impaired first-phase insulin secretion. However, populations with high rates of obesity-related NIDDM, such as the Pima Indians, tend to be more hyperinsulinemic[11,12] and have greater first-phase insulin responses compared with whites.[5]

Previous cross-sectional studies in Pima Indians have shown that the first-phase insulin response to glucose is reduced in IGT as compared with normal glucose tolerance, although longitudinally the transition from normal glucose tolerance to IGT showed only equivocal decreases.[3] Also, the relationship between plasma glucose and insulin concentrations during the oral glucose tolerance test (OGTT) remains normal in Pima Indians with IGT.[3] Therefore, the specific nature and timing of the onset of secretory impairment remains uncertain. In particular, to what degree is the first-phase insulin response to glucose impaired in IGT, and are any other aspects of insulin secretion reduced in relation to insulin action in IGT or mild NIDDM? To pursue these questions further, we examined in vivo β-cell function in more depth at the critical stages of IGT and early NIDDM.

SUBJECTS AND METHODS

Subjects

The subjects (Table 1) were Pima Indians living in the Gila River Indian Community in Arizona. They were participating in a longitudinal study of the development of NIDDM and obesity. Subjects were in good health as assessed by medical history, physical examination, and routine tests. All subjects gave informed consent, and the studies were approved by the ethics committees of the National Institutes of Health, the Indian Health Service, and the Tribal Council of the Gila River Indian Community. During the study (approximately 2 weeks in duration), subjects lived on a metabolic ward and were fed a weight-maintenance diet (50% of calories as carbohydrate, 30% as fat, and 20% as protein). Body composition was estimated by underwater weighing.[13,14] A minimum of 2 days after admission, all subjects underwent a 75-g OGTT, and glucose tolerance was assessed according to World Health Organization criteria.[15] Diabetic subjects were selected if

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Table 1. Physical Characteristics of Subjects

	Glucose Tolerance Status		
	Normal	Impaired	Diabetic
No. of subjects (M/F)	13/7	6/3	0/7
Age (yr)			
Mean ± SEM	28.3 ± 1.3	32.4 ± 1.7	29.8 ± 1.9
Range	20.3-40.0	25.0-40.0	22.3-38.3
Weight (kg)			
Mean ± SEM	100.8 ± 6.1	104.0 ± 6.3	93.2 ± 7.3
Range	55.6-144.7	87.8-144.3	66.2-114.6
Body fat (%)			
Mean ± SEM	33 ± 2	37 ± 2	41 ± 2
Range	11-44	29-47	34-47
Body mass index (kg/m^2)			
Mean ± SEM	36 ± 2	38 ± 2	38 ± 3
Range	21-49	31-48	28-48
Waist to thigh circumference			
Mean ± SEM	1.68 ± 0.03	1.69 ± 0.05	1.66 ± 0.08
Range	1.42-2.12	1.47-1.93	1.35-2.0

their fasting glucose was less than 7.8 mmol/L and they were on no medications.

Hyperinsulinemic, Euglycemic Clamp

After 8 to 15 days, a two-step hyperinsulinemic, euglycemic clamp was performed using a modification of the method reported by DeFronzo et al[16] as previously described.[17] Briefly, plasma insulin concentration is increased by means of a primed-continuous infusion of insulin, and glucose is infused to maintain plasma glucose at the basal level. The rate of glucose uptake is therefore a measure of insulin action. Two infusions of insulin (purified pork insulin, Velosulin; Novo Nordisk, Bethesda, MD) were administered consecutively, each for 100 minutes, at doses of 40 and 400 mU/m^2 body surface area. These resulted in mean ± SEM steady-state insulin concentrations of 1,070 ± 42 (coefficient of variation [CV], 4.1% ± 0.7%) and 18,500 ± 704 pmol/L (CV, 5.7% ± 0.7%), with the latter infusion inducing close to maximal insulin stimulation. The mean ± SEM glucose concentrations during the low- and high-dose infusions were 5.23 ± 0.04 mmol/L (CV, 2.1% ± 0.1%) and 5.27 ± 0.04 (CV, 2.6% ± 0.1%), respectively. There were no significant differences between the groups in these insulin and glucose concentrations.

Before and during the low-dose infusion, tracer amounts of 3-^{3}H-glucose were infused for 220 minutes to permit the calculation of the rate of glucose disappearance (equivalent to hepatic glucose output).[18] The quantification of hepatic glucose output using such single-compartment models has limitations, as previously described,[19] and negative values for hepatic glucose output during the low-dose insulin infusion in three normal subjects were assigned a value of zero. Nonparametric analysis (see later) was used to minimize these potential confounding effects.

Insulin-mediated glucose disposal at submaximally and maximally stimulating insulin concentrations were calculated as previously described.[5,17] The effects of variations in plasma glucose concentrations were adjusted to a plasma glucose level of 5.5 mmol/L as suggested by Best et al.[20] Differences in insulin concentrations between individual subjects during the low-dose insulin infusion were taken into account in the calculation of glucose uptake, as previously described.[21] Glucose uptake rates were normalized to estimated body size ([EMBS] = fat-free body mass + 20 kg), since metabolic rate is not directly proportional to fat-free mass.[22] The correct adjustment of glucose disposal rates for body size, glucose concentrations, and insulin concentrations during the clamp is debated, and therefore, statistical analyses were also performed on the glucose disposal values uncorrected for glucose and insulin concentrations and expressed by surface area rather than EMBS. The overall relationships between the groups remained the same in these analyses.

Three-Step Glycemic Clamp With Arginine

The three-step glycemic clamp as developed by Ward et al[23] was performed a minimum of 2 days after the euglycemic clamp. The acute insulin response (AIR_{arg}) to a maximally stimulating dose of 5 g arginine (Kabi Vitrum, Alameda, CA) was calculated (mean incremental insulin concentration from 3 to 5 minutes after the arginine bolus) at three levels of glycemia: fasting, midpoint following an infusion of glucose, and highest point following a glucose bolus and 30-minute infusion. There was a 2-hour washout period between the last two stages to avoid the priming effects of glucose.[24,25] Prestimulus glucose and insulin concentrations are listed in Table 2.

The magnitude of AIR_{arg} increases with increasing glycemia to a maximum at a glycemia of approximately 25 mmol/L.[23] Therefore, a dose-response curve for insulin secretion in response to glucose can be constructed using AIR_{arg} values at the three corresponding levels of glycemia. AIR_{arg} at the highest glycemia (AIR_{argMAX}) is a measure of β-cell capacity. In one subject with IGT and two subjects with NIDDM, AIR_{arg} at the highest glycemia was lower than that at the midpoint glycemia, so for the purposes of group comparisons, AIR_{arg} at the midpoint glycemia has been called AIR_{argMAX} in these subjects. The relationship between glucose concentration and AIR_{arg} has been shown to be linear over a glucose range of approximately 5 to 14 mmol/L,[26] and this allows a glucose potentiation slope for insulin secretion to be calculated as the change in AIR_{arg} divided by the change in plasma glucose between the fasting and midpoint levels of glycemia. The plasma glucose concentration at 50% AIR_{argMAX} (PG_{50}) can be used as an index of β-cell sensitivity, but in this study we have not used this measure because of the subjects whose measured maximum response was at the midpoint glycemia.

To compare AIR_{arg} between groups, AIR_{arg} at fasting glycemia was adjusted to a common basal glycemia of 5.5 mmol/L ($AIR_{arg\text{-}BAS}$) using the potentiation slope. These adjustments are therefore based on each individual's dose-response relationship and have been performed to ensure that any differences in AIR_{arg} are not due to differences in glycemia, which is an important potentiator of AIR_{arg}.

Intravenous Glucose Tolerance Test

The AIR to glucose (AIR_{glu}) was measured as the mean incremental insulin concentration from 3 to 5 minutes after an intravenous bolus of 25 g 50% dextrose. One NIDDM subject with

Table 2. Prestimulus Insulin Concentrations (pmol/L) During the Three-Step Glycemic Clamp With Arginine

	Glucose (mmol/L)	Glucose Tolerance		
		Normal	Impaired	Diabetic
Fasting glycemia	5.6 ± 0.5	239 ± 108	317 ± 107	377 ± 90*
Midpoint glycemia	10.4 ± 0.5	699 ± 514	740 ± 366	530 ± 195
Highest glycemia	29.2 ± 1.1	2,078 ± 1,024	2,186 ± 1,527	1,671 ± 998

NOTE. Mean ± SD.
*$P < .01$ *v* normal.

a negative response was assigned a value of 50 pmol/L so that log-transformation could be performed.

OGTT

A standard 75-g OGTT was performed with samples for glucose and insulin drawn at 0, 30, 60, 120, and 180 minutes. Mean OGTT glucose and insulin concentrations were calculated as the area under the curve divided by 180 minutes.

Glucose and Insulin Measurements

Plasma glucose concentrations were measured by the glucose oxidase method using the Beckman analyzer (Beckman Instruments, Fullerton, CA). Plasma insulin concentrations were determined by a modification[27] of the radioimmunoassay reported by Yalow and Berson[28] for all tests except for the glycemic clamp test with arginine, where a Concept 4 analyzer (Micromedic, Horsham, PA) was used. Each statistical comparison between groups was made using one assay method only, so that systematic differences between methodologies would not affect the analyses. However, for visual comparisons of insulin concentrations between the arginine test and the other tests, a correction factor derived from multiple duplicate testing by these two methods was applied to the Concept 4 insulin values ([C4-ins]) to make them comparable to the other insulin results: (insulin = 0.8546 + 0.4681 · log[C4-ins] + 0.0989 · log[C4-ins]^2).

Statistical Methods

Statistical analyses were performed with the procedures of the SAS Institute (Cary, NC). Data shown are arithmetic. Statistical analysis was made with nonparametric methods (Kruskal-Wallis test for comparing all three groups, and the Wilcoxon two-sample test for comparing two groups) because many of the insulin-derived variables were not normally distributed and the potential errors of the tracer methodology used[19] may have meant that hepatic glucose output calculations were quantitatively in error, although the ranking of subjects was less likely to be affected. Insulin-derived variables and glucose disposal at submaximal insulinemia were log-transformed for the correlation calculations (Pearson correlations) and for the calculation of linear regression models for Fig 2. The effects of glucose tolerance status were tested in the log models, and the data were then back-transformed for presentation in Fig 2. Glucose adjustment of the mean insulin concentration during the OGTT was made using two methods: a simple ratio between insulin and glucose concentrations was calculated (insulin to glucose ratio), and the residual insulin values from a linear model (log insulin concentration = glucose concentration) were added to the group mean for the logged insulin values and then retransformed to arithmetic data (glucose-adjusted). Data are the mean ± SEM unless otherwise indicated.

RESULTS

Subjects

There were differences in body fat between the groups, although these did not reach statistical significance ($P = .07$, ANOVA). The higher percent body fat in the NIDDM group was at least in part due to the absence of males, whose body composition is generally lower in fat than females. The normal group, on average, was considerably overweight (body fat of 33% and body mass index of 36), but a wide range of obesity was represented (11% to 44% body fat).

OGTT

The NIDDM group had only mild diabetes, with mean fasting and 2-hour glucose concentrations of 6.3 ± 0.3 and 13.7 ± 9.7 mmol/L. The mean OGTT insulin concentration (comparable to the area under the curve) increased significantly with glucose tolerance status, and this reflected differences in the 120- and 180-minute values. The 30- and 60-minute insulin values, which tend to be less glucose-dependent, were not different between groups. There were no differences between groups once the insulin concentrations were adjusted for glucose (Table 3).

Basal Hepatic Glucose Output and Insulin Action

Basal hepatic glucose output was similar in all three groups. Since the majority of glucose uptake under basal conditions is directed mainly to insulin-independent tissues, normalization to metabolic size may not be appropriate. However, basal hepatic glucose output in absolute terms before normalization to body size was still similar between groups (mean ± SD: normal 185 ± 32, IGT 175 ± 24, and NIDDM 153 ± 24 mg/min). Glucose disposal rates at submaximally and maximally stimulating insulin concentrations were lower than normal in the IGT ($P < .05$ and $P < .02$, respectively) and NIDDM ($P < .005$ for both) groups, but they were not significantly different between IGT and NIDDM (Table 4).

Insulin Secretion

The NIDDM group had lower AIR_{glu} as compared with the normal ($P < .0001$) and IGT ($P < .001$) groups, $AIR_{arg\text{-}BAS}$ was not significantly different in those with NIDDM (Table 5). The insulin secretory capacity (AIR_{argMAX}) was not significantly different ($P = .09$) across all three groups, but the standard error of the IGT group was approximately twice that of the other groups, reflecting the wide variation of responses in this group (Fig 1). The NIDDM group had a lower AIR_{argMAX} as compared with the normal group ($P < .05$). The glucose potentiation slope showed a borderline significant effect across the groups ($P = .05$), with the diabetic group being lower than both normal and IGT groups ($P < .05$). Individual data points from the three-

Table 3. OGTT Glucose and Insulin Concentrations

	Glucose Tolerance Status		
	Normal	Impaired	Diabetic
Glucose (mmol/L)			
Fasting	4.9 ± 0.1	5.4 ± 0.2	6.3 ± 0.3
2-hour	6.5 ± 0.2	9.7 ± 0.4	13.7 ± 0.7
Mean concentration	6.6 ± 0.1	8.8 ± 0.3	11.7 ± 0.4
Insulin (pmol/L)			
Fasting*	246 ± 26	358 ± 56	521 ± 85
Mean concentration†	1,238 ± 146	1,849 ± 348	1,953 ± 280
Mean concentration (glucose-adj)	1,536 ± 139	1,718 ± 318	1,269 ± 255

NOTE. Mean ± SEM.

*$P < .01$ Kruskal-Wallis test for effect of glucose tolerance status: normal *v* NIDDM, $P < .01$.

†$P < .05$ Kruskal-Wallis test for effect of glucose tolerance status: normal *v* NIDDM, $P < .05$.

Table 4. Basal Hepatic Glucose Output and Insulin Action During a Euglycemic Clamp

	Glucose Tolerance		
	Normal	Impaired	Diabetic
Basal hepatic glucose output (mg/kg EMBS · min)*	2.16 ± 0.05	2.05 ± 0.05	2.05 ± 0.05
Glucose disposal at submaximum insulinemia (mg/kg EMBS · min)†	2.74 ± 0.21	2.11 ± 0.12	1.85 ± 0.05
Glucose disposal at maximum insulinemia (mg/kg EMBS · min)‡	8.72 ± 0.37	6.84 ± 0.63	6.15 ± 0.59

NOTE. Mean ± SEM.

*EMBS = estimated metabolic body size (fat-free mass + 20 kg).

†Effect of glucose tolerance status (Kruskal-Wallis) $P < .05$. IGT ($P < .05$) and NIDDM ($P < .005$) *v* normal.

‡Effect of glucose tolerance status (Kruskal-Wallis) $P < .002$. IGT ($P < .02$) and NIDDM ($P < .005$) *v* normal.

step glycemic clamp for IGT and NIDDM subjects as compared with the mean and 95% confidence intervals for the normal group are shown in Fig 1.

Relationship Between Insulin Secretion and Insulin Action

To determine whether IGT or NIDDM groups had a secretory deficit relative to the degree of insulin resistance, we examined the relationship between the insulin secretion parameters (AIR_{glu}, AIR_{argBAS}, AIR_{argMAX}, and potentiation slope) and glucose disposal rates at submaximally stimulating insulin concentrations (Fig 2A to D). All three groups were represented at the insulin-resistant end of the range, but the insulin-sensitive end of the range (>3 mg/kg EMBS · min) was totally dominated by normal subjects.

Compared with the relationship in normals between glucose disposal and insulin secretion, the IGT group were not different. Subjects with IGT mostly remained in the normal range, but seven of nine were below the regression line in Fig 2A, suggesting that a relatively low AIR_{glu} may be present in some subjects with IGT. In each instance, the NIDDM group demonstrated a relative insulin deficiency (low insulin secretion for the degree of insulin action, $P < .01$ to $P < .0001$). Similarly, in relation to glucose disposal rates at maximally stimulating insulin concentrations, the NIDDM group had decreased AIR_{glu} ($P < .0001$), AIR_{argBAS} ($P < .05$), AIR_{argMAX} ($P < .01$), and potentiation slope ($P = .05$) as compared with normals, whereas the IGT group was not different from normals.

While the wide range of insulin sensitivity in normal subjects contributed to the derivation of the normal regression line, it meant that the groups were not well-matched for degree of insulin sensitivity. Another approach to the important question of whether insulin secretion is impaired in the IGT group could be to exclude the five insulin-sensitive normal subjects (glucose disposal at submaximal insulinemia >3 mg/kg EMBS · min) and compare insulin secretion variables between groups. Although this imposed similar degrees of insulin sensitivity in a post hoc and somewhat arbitrary fashion, it did improve the matching of the groups. Under these conditions, this more insulin-resistant but normal glucose-tolerant group (now n = 15 and mean body fat = 37%) had similar glucose disposal

Table 5. Insulin Secretion in Pima Indians With Normal, Impaired, or Diabetic Glucose Tolerance

	Glucose Tolerance Status		
Parameter	Normal (n = 20)	Impaired (n = 9)	Diabetic (n = 7)
First-phase secretion (pmol/L, 3 to 5 min)			
AIR_{glu}*	1,878 ± 220	1,748 ± 273	280 ± 133
AIR_{arg}	1,261 ± 132	1,717 ± 279	1,157 ± 135
AIR_{argBAS}†	1,282 ± 124	1,608 ± 287	1,050 ± 182
AIR_{argMAX}‡ (pmol/L, 3 to 5 min)	5,589 ± 484	6,531 ± 1,205	3,619 ± 651
Potentiation slope§ (pmol/L insulin/mmol/L glucose)	456 ± 48	582 ± 149	279 ± 52

NOTE. Mean ± SEM.

*Effect of glucose tolerance status (Kruskal-Wallis) $P < .001$. Normal ($P < .001$) and IGT ($P < .01$) *v* NIDDM.

†Adjusted to basal glucose of 5.5 mmol/L.

‡Effect of glucose tolerance status (Kruskal-Wallis) $P = .09$. Normal *v* NIDDM $P < .05$.

§Effect of glucose tolerance status (Kruskal-Wallis) $P = .05$. Normal ($P < .05$) and IGT ($P < .05$) *v* NIDDM.

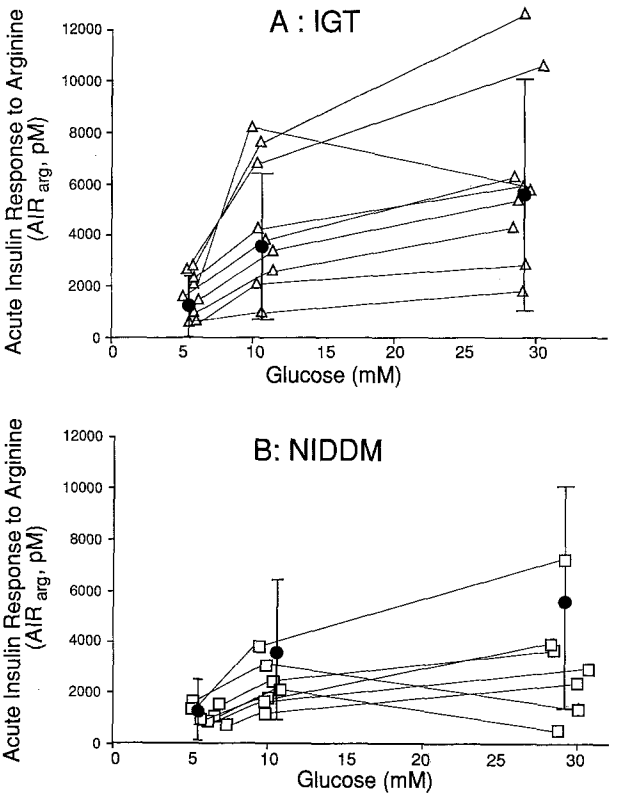

Fig 1. AIR_{arg} as a function of glucose concentration. The arithmetic mean and 95% confidence intervals for the 20 subjects with normal glucose tolerance (●) is shown in both graphs, along with the individual plots of (A) 9 subjects with impaired glucose tolerance (△) and (B) 7 subjects with mild NIDDM (□).

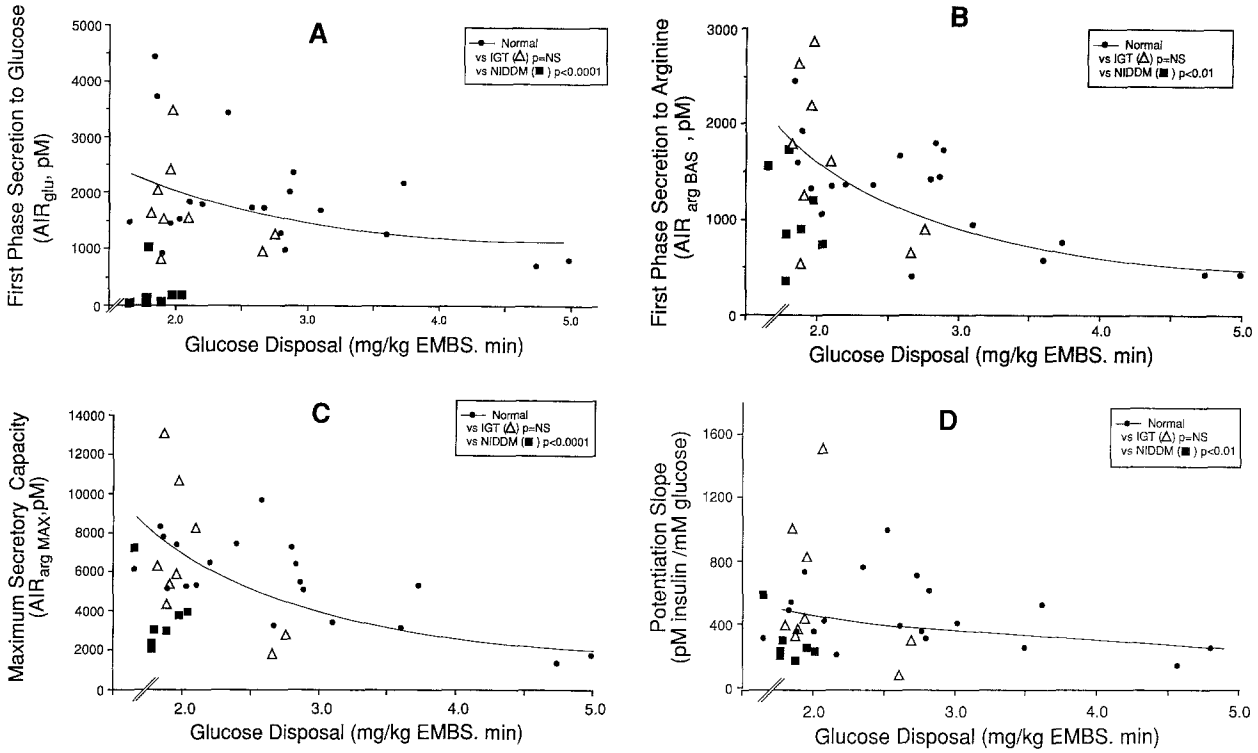

Fig 2. Relationship between insulin secretion parameters and insulin action. Glucose disposal rate at submaximally stimulating insulin concentrations (on the X axis in each case) is plotted against AIR_{glu} (A), AIR_{arg} (B), maximal secretory capacity (C), and potentiation slope of glucose for insulin secretion (D). The regression line was calculated from subjects with normal glucose tolerance (●). Subjects with IGT (△) were not significantly different from normals.

rates at submaximal insulinemia. However, this subgroup of normal subjects still had a higher glucose disposal at maximal insulinemia ($P < .03$) as compared with the IGT group, and the new means for AIR_{glu} (2,056 ± 266 pmol/L), AIR_{argBAS} (1,499 ± 115 pmol/L), AIR_{argMAX} (6,441 ± 412 pmol/L), and potentiation slope (502 ± 56) were not significantly different from those of the IGT group shown in Table 5.

Correlation Analysis With OGTT

Simple correlation analysis showed that glucose tolerance, defined as the mean glucose concentration during the OGTT, was inversely correlated with insulin action at submaximally and maximally stimulating insulin concentrations ($r = -.66, P < .001$ and $r = -.65, P < .0001$, respectively). AIR_{glu} was inversely correlated with glucose tolerance ($r = -.66, P < .0001$), but this was entirely due to the presence of the low responses in the NIDDM group. The other secretion parameters (AIR_{argBAS}, AIR_{argMAX}, and potentiation slope) did not correlate with glucose tolerance when all subjects or only nondiabetic subjects were considered. In a multiple regression model using all subjects, insulin action ($P < .0002$) and AIR_{glu} ($P < .0001$) were the only significant determinants of glucose tolerance. The models using AIR_{glu} and insulin action at submaximally or maximally stimulating insulin concentrations explained 63% and 72% of the variance in glucose tolerance, respectively.

Correlations with the insulin response to oral glucose were calculated (Table 6) using the unadjusted insulin values, the insulin to glucose ratio, and insulin values adjusted for glucose by the residuals method. The correlations shown are for the mean insulin concentration through-

Table 6. Pearson Correlation Coefficients (and *p* values) With the Insulin Response to Oral Glucose

Parameter	Mean Insulin Concentration during OGTT		
	Unadjusted	Ins/Glu Ratio	Glucose-adj*
Glucose tolerance (mean OGTT concentration)	.53 (<.0002)	—	—
Insulin Action†			
Glucose disposal at submaximal insulinemia	−.72 (<.0001)	−.43 (<.01)	−.50 (<.01)
Glucose disposal at maximal insulinemia	−.61 (<.0001)	−.34 (<.05)	−.32 (=.06)
Insulin secretion			
AIR_{glu}	.03 (NS)	.38 (<.05)	.44 (<.01)
AIR_{argBAS}‡	.62 (<.0001)	.67 (<.0001)	.75 (<.0001)
AIR_{argMAX}	.44 (<.01)	.53 (<.001)	.61 (<.0001)
Potentiation slope	.32 (=.06)	.46 (<.01)	.49 (<.01)

*Glucose adjustment: calculated from the residuals of the insulin glucose relationship.

†Euglycemic clamp at submaximally and maximally stimulating insulin concentrations.

‡Corrected to a glucose of 5.5 mmol/L.

out the OGTT (equivalent to the area under the curve), but the 2-hour insulin concentrations gave similar results. Unadjusted insulin concentrations were positively correlated with glucose concentration ($P < .001$), negatively correlated with insulin action ($P < .001$), and positively correlated with some of the insulin secretion indices. Adjustment for glucose concentration either using the insulin to glucose ratio or the residuals method tended to diminish the strength of the relationships with insulin action and enhance the correlations with insulin secretion.

DISCUSSION

In this study, we have measured insulin secretion in Pima Indians with normal, impaired, or mildly diabetic glucose tolerance using several discriminating in vivo tests. The aim was to define more specifically the nature and timing of the secretory impairment in glucose-intolerant subjects from this diabetes-prone population. We found that subjects with IGT were more insulin-resistant as compared with normals, but a wide range of all insulin secretory responses was measured (as reflected in the large standard errors). Although there was a suggestion that some IGT subjects with moderate insulin resistance also had impaired AIR_{glu}, as a group, no significant secretory defects were demonstrated. By contrast, those with early NIDDM displayed both insulin resistance and secretory impairment: AIR_{glu}, in particular, was absolutely impaired, but all aspects of insulin secretion were impaired relative to the degree of insulin resistance.

The pattern of secretory decline in Pima Indians is important because it may reflect the usual pattern seen in ethnic groups with high rates of NIDDM. A reduction in the AIR_{glu} has been previously described in NIDDM and in some instances IGT.[3,7-10] While the current study has not duplicated these findings for IGT, this may simply reflect the small numbers of IGT subjects compared with the wide variation in responses in insulin secretion. However, the study has shown, that the other measures of insulin secretion are less affected than AIR_{glu} in mild NIDDM, and that in IGT they appear normal. Other more subtle aspects of insulin secretion, such as the loss of the normal oscillation pattern,[29] may be present in Pima Indians with IGT, but were not assessed in this study.

Although the groups were not ideally matched for sex or body size, this should not alter the overall interpretation of the results. The absence of males in the mild NIDDM subjects meant that this group had a slightly higher percent body fat than the IGT group for the same mean body mass index. This represents normal sex differences in body composition, and there is no reason to believe that there are sex differences in the metabolic processes leading to NIDDM. The wide range of body fat in the normal group allowed better models of the normal relationship between insulin action and insulin secretion; however, analyses excluding insulin-sensitive (mainly leaner) normal subjects did not alter the results.

Although this is a cross-sectional study, the implication of these findings is that the loss of AIR_{glu} may be the critical event in the transition from IGT, where it is largely intact in absolute terms, to early NIDDM, where it is significantly depressed. Furthermore, since other aspects of insulin secretion were less affected at this early stage of NIDDM, especially the first-phase response to the nonglucose secretagogue, arginine, the initial secretory defect may be at the level of glucose sensing for first-phase release. This has been previously postulated from studies of acute-phase insulin secretion.[30,31]

Both animal studies and clinical evidence support the concept that glucose itself may be toxic to β cells, such that β-cell overstimulation leads to desensitization and eventually insulin secretory failure.[32-35] In light of this concept, the current results suggest that reduced first-phase glucose sensing might indicate the site of a specific biochemical lesion, which could result in genetically susceptible Pimas developing β-cell decompensation and diabetes mellitus.

The assessment of secretory capacity (AIR_{argMAX}) and potentiation slope was made from the dose-response curve for glucose potentiation of AIR_{arg} during a three-level glycemic clamp. In some subjects (two IGT and one NIDDM), AIR_{arg} at the highest glycemia was less than at the midpoint glycemia (Fig 1). This phenomenon was not reported in the previous use of this test in subjects with established NIDDM,[23] but in that study, AIR_{arg} at five levels of glycemia was assessed over 2 days. We interpret this lack of a sequential increase in AIR_{arg} as a sign of early β-cell failure, which could be due to exhaustion or enhanced desensitization.[32]

The reason some subjects have IGT may not be self-evident when they seem to have a relatively normal relationship between insulin secretion and insulin action. An undetected impairment of insulin secretion may be one explanation, but the normal consequences of a feedback system may be another. The assumptions for this are that insulin resistance and insulin secretion are linked by a negative-feedback control loop that involves glucose concentration as the signal. The normal close matching of insulin secretion to insulin action (Kahn et al[41] and Fig 2A to D) supports this relationship, and glucose would be an obvious signal. Thus, in the face of increasing insulin resistance, the increasing secretory rates are only maintained by the signal of increasing glycemia, even to the point of glycemia in the IGT range if the insulin resistance is severe enough.

From the current data and previous studies on the natural history of NIDDM in this population,[2-5,36-38] the following sequence of events in the evolution of NIDDM in the Pimas seems likely: Insulin resistance develops because of an underlying genetic predisposition,[17,39] obesity,[22] or other causes,[40] and a compensatory increase in insulin secretion occurs.[41] Mild hyperglycemia is required to maintain this increase in insulin secretion. IGT develops if the insulin resistance is so great that the glycemia needed to maintain appropriately high insulin secretion is in the IGT range. Since seven of nine subjects with IGT showed a relatively low AIR_{glu}, it is possible that AIR_{glu} has already started to decline or has reached an inherently low limit of adaptation in some of these subjects.[7-10] The onset of early NIDDM occurs in the presence of severe insulin resistance but coincides with an absolute decline in AIR_{glu} and a

relative decline in other aspects of insulin secretion. Late NIDDM with a high fasting glucose occurs when, in addition to the severe insulin resistance, all aspects of insulin secretion are further decreased and basal hepatic glucose production is increased.[2]

Extrapolation of the current findings in Pima Indians to other racial groups should be made with caution, since it has been shown that Pimas have greater insulin secretion (AIR_{glu}, AIR_{argMAX}, and potentiation slope) than whites at all levels of insulin action.[3,42] Also, in other racial groups, IGT may be more commonly associated with impaired insulin secretion[10] with only mild insulin resistance.

Longitudinal studies in subjects with normal glucose tolerance have shown that insulin resistance predicts the development of NIDDM.[38] In subjects who already have IGT, a low insulin response relative to glucose concentration after a glucose load is predictive of conversion from IGT to NIDDM.[36,43,44] These low OGTT insulin concentrations seen in epidemiologic studies are assumed to reflect impaired insulin secretion, although this has not been validated in the IGT and early-NIDDM stages. In this study, we have shown that, provided adjustment is made for glucose concentration, insulin concentration during an OGTT can be a fair reflection of insulin secretory function, although the correlation between insulin action and adjusted insulin concentrations still remains. Glucose adjustment by the residuals method (residual values from the linear model log insulin secretion = glucose concentration) is probably the more technically correct method, but it cannot be applied to groups that include diabetic subjects with high fasting glycemia, since the relationship between glucose and insulin concentrations then becomes nonlinear.[3]

In summary, we have found that in Pima Indians subjects with IGT were mainly differentiated from normals by increased insulin resistance, and that no significant abnormality of insulin secretion was found, although a relative impairment of AIR_{glu} in IGT could not be excluded in this study. Subjects with mild NIDDM were no more insulin-resistant than those with IGT, but they did have decreased insulin secretion both absolutely (AIR_{glu}) and relative to the degree of insulin resistance (all secretion indices) as compared with normals. The loss of glucose sensing for first-phase insulin secretion may be the critical event in the transition from IGT to early NIDDM.

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REFERENCES

1. Knowler WC, Pettitt DJ, Bennett PH, et al: Diabetes mellitus in the Pima Indians: Genetic and evolutionary considerations. Am J Phys Anthropol 62:107-114, 1983

2. Defronzo RA: The triumvirate: B-Cell, muscle, liver. A collusion responsible for NIDDM. Diabetes 37:667-687, 1988

3. Lillioja S, Mott DM, Howard BV, et al: Impaired glucose tolerance as a disorder of insulin action. Longitudinal and cross-sectional studies in Pima Indians. N Engl J Med 318:1217-1225, 1988

4. Saad MF, Knowler WC, Pettitt DJ, et al: A two-step model for development of non-insulin diabetes. Am J Med 90:229-235, 1991

5. Lillioja S, Nyomba BL, Saad MF, et al: Exaggerated early insulin release and insulin resistance in a diabetes-prone population: A metabolic comparison of Pima Indians and caucasians. J Clin Endocrinol Metab 73:866-870, 1991

6. Bogardus C, Lillioja S, Howard BV, et al: Relationships between insulin secretion, insulin action and fasting plasma glucose concentrations in non-diabetic and non–insulin dependent diabetic subjects. J Clin Invest 74:1238-1246, 1984

7. Mitrakou M, Kelley D, Mokan M, et al: Role of reduced suppression of glucose production and diminished early insulin release in impaired glucose tolerance. N Engl J Med 326:22-29, 1992

8. Cook JTE, Page RCL, Levy JC, et al: Hyperglycaemic progression in subjects with impaired glucose tolerance: Association with decline in beta cell function. Diabetic Med 10:321-326, 1993

9. Ratzmann KP, Schulz B, Heinke P, et al: Quantitative and qualitative changes in early insulin response to glucose in subjects with impaired carbohydrate tolerance. Diabetes Care 4:85-91, 1981

10. Kanatsuka A, Makino H, Sakurada M, et al: First-phase response to glucose in non-obese or obese subjects with glucose intolerance: Analysis by C-peptide secretion rate. Metabolism 37:878-884, 1988

11. O'Dea K, Traianedes K, Hopper JL, et al: Impaired glucose tolerance, hyperinsulinemia, and hypertriglyceridemia in Australian Aborigines from the desert. Diabetes Care 11:23-29, 1988

12. Hafner SM, Stern MP, Hazuda HP, et al: Hyperinsulinemia in a population at high risk for non–insulin-dependent diabetes mellitus. N Engl J Med 315:220-224, 1990

13. Goldman RF, Buskirk ER: A method for underwater weighing and the determination of body density, in Brozek J, Herschel A (eds): Techniques for Measuring Body Composition. Washington, DC, National Academy of Science, National Research Council, 1961, pp 78-106

14. Keys A, Brozek J: Body fat in adult man. Physiol Rev 33:245-325, 1953

15. Report of a WHO Study Group: Diabetes mellitus. WHO Tech Rep Ser 727:10-14, 1985

16. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: A method for quantifying insulin resistance. Am J Physiol 237:E214-E223, 1979

17. Lillioja S, Mott DM, Zawadzki JK, et al: In vivo insulin action is familial characteristic in non-diabetic Pima Indians. Diabetes 36:1329-1335, 1987

18. Steele R: Influences of glucose loading and of injected insulin on hepatic glucose output. Ann NY Acad Sci 82:420-430, 1959

19. Yki-Jarvinen H, Consoli A, Nurjhan N, et al: Mechanism for underestimation of isotopically determined glucose disposal. Diabetes 38:744-751, 1989

20. Best JD, Taborsky GT Jr, Halter JB, et al: Glucose disposal is not proportional to plasma glucose level in man. Diabetes 30:847-850, 1981

21. Gottesman I, Mandarino L, Gerich J: Estimation and kinetic analysis of insulin-independent glucose uptake in human subjects. Am J Physiol 244:E632-E635, 1983

22. Lillioja S, Bogardus C: Obesity and insulin resistance: Lessons learned from the Pima Indians. Diabetes Metab Rev 4:517-540, 1988

23. Ward WK, Bolgiano DC, McKnight B, et al: Diminished B-cell secretory capacity in patients with non–insulin dependent diabetes mellitus. J Clin Invest 74:1318-1328, 1984

24. Cerasi E: Potentiation of insulin release by glucose in man. Acta Endocrinol (Copenh) 79:483-501, 1975

25. Grill V: Time and dose dependencies for priming effect of glucose on insulin secretion. Am J Physiol 240:E24-E31, 1981

26. Beard JC, Ward WK, Halter JB, et al: Relationship of islet function to insulin action in human obesity. J Clin Endocrinol Metab 65:59-64, 1987

27. Herbert V, Lau K, Gottlieb CW, et al: Coated charcoal immunoassay of insulin. J Clin Endocrinol Metab 25:1375-1384, 1965

28. Yalow RS, Berson SA: Immunoassay of endogenous plasma insulin in man. J Clin Invest 39:1157-1167, 1960

29. O'Meara NM, Sturis J, Van Cauter E, et al: Lack of control by glucose of ultradian insulin secretory oscillation in impaired glucose tolerance and in non–insulin dependent diabetes mellitus. J Clin Invest 92:262-271, 1993

30. Simpson RG, Benedetti A, Grodsky GM, et al: Early phase of insulin release. Diabetes 17:684-692, 1968

31. Palmer JP, Benson JW, Walter RM, et al: Arginine-stimulated acute phase of insulin and glucagon secretion in diabetic subjects. J Clin Invest 58:565-570, 1976

32. Grodsky GM: A new phase of insulin secretion. How will it contribute to our understanding of B-cell function? Diabetes 38:673-678, 1989

33. Seltzer HS, Harris VL: Exhaustion of insulogenic reserve in maturity-onset diabetic patients during prolonged and continuous hyperglycemic stress. Diabetes 13:6-13, 1964

34. Leahy JL, Bonner-Weir S, Weir GC: Minimal chronic hyperglycemia is a critical determinant of impaired insulin secretion after an incomplete pancreatectomy. J Clin Invest 81:1407-1414, 1988

35. Unger RH, Grundy S: Hyperglycemia as an inducer as well as a consequence of impaired islet cell function and insulin resistance. Implications for the management of diabetes. Diabetologia 28:119-121, 1985

36. Saad MF, Knowler WC, Pettitt DJ, et al: The natural history of impaired glucose tolerance in the Pima Indians. N Engl J Med 319:1500-1506, 1988

37. Saad MF, Knowler WC, Pettitt DJ, et al: Sequential changes in serum insulin concentrations during development of non–insulin dependent diabetes mellitus. Lancet 1:1356-1359, 1989

38. Lillioja S, Mott DM, Spraul M, et al: Insulin resistance and insulin secretory dysfunction as precursors of non–insulin dependent diabetes mellitus. Prospective studies of Pima Indians. N Engl J Med 329:1988-1992, 1993

39. Bogardus C, Lillioja S, Nyomba BL, et al: Distribution of in vivo insulin action in Pima Indians as a mixture of three normal distributions. Diabetes 38:1423-1432, 1989

40. Mott DM, Lillioja S, Bogardus C: Overnutrition-induced decrease in insulin action for glucose storage: In vivo and in vitro in man. Metabolism 35:160-165, 1986

41. Kahn SE, Beard JC, Schwartz MW, et al: Increased β-cell secretory capacity as mechanism for islet adaptation to nicotinic acid–induced insulin resistance. Diabetes 38:562-568, 1989

42. Swinburn BA, Bogardus C: Equivalent insulin resistance, but greater insulin secretion in Pima Indians compared to caucasians. Proc Aust Diabetes Soc 27:27, 1990 (abstr)

43. Sicree RA, Zimmet PZ, King HO, et al: Plasma insulin response among Nauruans. Prediction of deterioration in glucose tolerance over 6 yr. Diabetes 36:179-186, 1987

44. Kadowaki T, Miyake Y, Hagura R, et al: Risk factors for worsening to diabetes in subjects with impaired glucose tolerance. Diabetologia 26:44-49, 1984