A High Fasting Plasma Insulin Concentration Predicts Type 2 Diabetes Independent of Insulin Resistance

Evidence for a Pathogenic Role of Relative Hyperinsulinemia

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Fasting hyperinsulinemia is a widely used surrogate measure of insulin resistance and predicts type 2 diabetes in various populations. Whether fasting hyperinsulinemia predicts diabetes independent of insulin resistance is unknown. In 319 Pima Indians with normal glucose tolerance, fasting plasma insulin concentration and insulin-stimulated glucose disposal (*M*) (hyperinsulinemic clamp) were inversely related, but at any given *M*, there was substantial variation, with some subjects being hyperinsulinemic and others being hypoinsulinemic relative to their degree of insulin sensitivity. In 262 of the 319 subjects followed prospectively over 6.4 ± 3.9 years, a high fasting plasma insulin concentration was a significant independent predictor of diabetes, in addition to low *M* and low acute insulin response (AIR) (intravenous glucose challenge). In 161 of the 319 subjects with follow-up measurements of *M* and AIR (5.1 ± 3.9 years), a high relative fasting plasma insulin concentration predicted a decline in AIR but not in *M* before the onset of diabetes. The adjusted fasting plasma insulin concentration was a familial trait (heritability of 0.52) and in a genome-wide scan, there was suggestive evidence of linkage (logarithm of odds score 1.77) to a region on chromosome 3q, which harbors the gene encoding *GLUT2*. These results provide the first prospective evidence in humans that fasting hyperinsulinemia itself has a primary role in the pathogenesis of diabetes, independent of insulin resistance. Whether amelioration of basal insulin hypersecretion will prevent diabetes remains to be elucidated.
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AIR, acute insulin response; EMBS, estimated metabolic body size; IGT, impaired glucose tolerance; LOD, logarithm of odds; *M*, insulin-stimulated glucose disposal; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test.

Insulin resistance and hyperinsulinemia are common abnormalities in individuals at high risk for type 2 diabetes, such as those with impaired glucose tolerance (IGT) (1–4) or first-degree relatives of individuals with type 2 diabetes (5,6). Numerous prospective studies have demonstrated the pathogenic importance of these abnormalities in the development of type 2 diabetes (7–22). Among Pima Indians with initially normal glucose tolerance (NGT), a low rate of insulin-stimulated glucose disposal, as assessed by the hyperinsulinemic-euglycemic clamp technique, predicts the development of diabetes (7). Prospective studies in other populations also suggest that insulin resistance predicts diabetes (8–22). In these studies, insulin sensitivity was not directly quantified, however (8–22). Instead, in most studies (11–22), insulin resistance was inferred from high fasting plasma insulin or C-peptide concentrations, which were consistently found to predict diabetes.

Although fasting hyperinsulinemia is a widely used surrogate measure of insulin resistance, variability in the fasting plasma insulin concentration is only partially explained by differences in insulin sensitivity (23). It is therefore unclear if insulin resistance is the only explanation for why fasting hyperinsulinemia predicts diabetes or whether fasting hyperinsulinemia may have a pathogenic role, independent of insulin resistance. There are several lines of evidence to suggest that fasting hyperinsulinemia itself may be a primary metabolic defect and not simply a secondary consequence of insulin resistance. First, experimental hyperinsulinemia established by pancreatic venous diversion in dogs (24) or by prolonged hyperinsulinemic clamps in humans (25) causes insulin resistance. Second, basal hypersecretion of insulin was found to be a more common abnormality than insulin resistance in a large group of nondiabetic obese Caucasians (26). Third, hypersecretion of insulin is one of the earliest detectable abnormalities in various animal models of type 2 diabetes (27–31), such as in rats with lesions of the ventromedial hypothalamus (28). In this model, amelioration of insulin hypersecretion by vagotomy largely delays or even prevents the development of diabetes (28). Finally, marked hyperinsulinemia is a common characteristic of several ethnic groups with a high prevalence of diabetes, such as Native-

Americans (7,32), Mexican-Americans (6,15), and Pacific Islanders (16). In Pima Indians, plasma insulin concentrations are increased at an early age (33,34) and are higher than those in Caucasians, even after adjusting for the higher degree of insulin resistance (32). Despite their hyperinsulinemia, Pima Indians have one of the highest reported prevalence rates of diabetes in the world.

Based on the above findings, it has been suggested that basal hypersecretion of insulin may be an independent abnormality in the pathogenesis of diabetes (26,27,34,35) and that in some populations, primary (not compensatory) hyperinsulinemia, rather than insulin resistance, may be the primary genetic defect (34,35). In the present series of studies, we tested this hypothesis by analyzing data from an ongoing study of the pathogenesis of type 2 diabetes in Pima Indians (7,36). First, we examined the relationship between the fasting plasma insulin concentration and insulin sensitivity in a large baseline population of subjects with NGT, assessed the determinants of fasting insulinemia, and identified individuals who were relatively hyper- and hypoinsulinemic for their degree of insulin sensitivity and adiposity. We then followed these subjects prospectively to test whether a high fasting plasma insulin concentration predicts diabetes independent of insulin resistance. Next, we tested whether individuals who were relatively hyperinsulinemic at baseline are predisposed to develop abnormalities in early-phase insulin secretion and/or insulin sensitivity before the onset of diabetes. Finally, we assessed whether relative hyperinsulinemia is a familial trait and undertook a genome-wide autosomal scan to identify chromosomal loci linked to this measure.

RESEARCH DESIGN AND METHODS

Subjects. Subjects in this study were participants in an ongoing longitudinal study of the pathogenesis of type 2 diabetes initiated in 1982 (7,36). Except for a small group of Caucasians recruited for cross-sectional comparisons, all participants were Pima (or closely related Tohono O'Odham) Indians from the Gila River Indian Community near Phoenix, Arizona. A total of 384 subjects (319 Pima Indians and 65 Caucasians) with NGT (Table 1) were admitted for 8–15 days to the Clinical Research Unit of the National Institutes of Health in Phoenix. After at least 3 days on a weight-maintaining diet, a series of tests were conducted to assess body composition, oral glucose tolerance, fasting plasma insulin concentration, insulin sensitivity, and early-phase insulin secretion (see below). The Pima Indian subjects were then invited back at approximately annual intervals for repeat oral glucose tolerance tests (OGTTs) and, in about two-thirds of the subjects, for repeat assessment of insulin sensitivity and early-phase insulin secretion. All subjects were healthy according to a comprehensive medical history, physical examination, and routine blood and laboratory tests, and none smoked or took medications known to alter glucose or insulin metabolism at the time of the study. The study protocol was approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases and by the Tribal Council of the Gila River Indian Community. All subjects gave written informed consent before participation.

Anthropometric measurements. Body composition was estimated by underwater weighing with determination of residual lung volume by helium dilution or by total body dual-energy X-ray absorptiometry (DPX-L; Lunar Radiation, Madison, WI) (7,36). Waist and thigh circumferences were measured at the umbilicus and the gluteal fold in the supine and standing position, respectively, and the waist-to-thigh ratio was calculated as an index of body fat distribution.

OGTT. After a 12-h overnight fast, subjects underwent a 75-g OGTT (37). Plasma samples were drawn at baseline and after 2 h for determination of plasma glucose and insulin concentrations. Glucose tolerance was classified according to the 1985 World Health Organization diagnostic criteria (37).

Hyperinsulinemic-euglycemic glucose clamp. Insulin sensitivity was assessed by a hyperinsulinemic-euglycemic glucose clamp as previously described (7,36). In brief, after an overnight fast, a primed continuous intravenous insulin infusion was administered for 100 min at a constant rate of 40 mU per square meter body surface area per minute, leading to a steady-state plasma insulin concentration of 140 ± 42 μU/ml (mean ± SD). Plasma glucose concentrations were maintained at ~100 mg/dl with a variable infusion of a 20% glucose solution. From the rate of glucose infused during the last 40 min of the clamp and the rate of endogenous glucose output (measured by a primed [30 μCi] continuous [0.3 μCi/min] [3-^{3}H]glucose infusion), the rate of total insulin-stimulated glucose disposal (M) was calculated, adjusted for steady-state plasma glucose and insulin concentrations, and normalized to estimated metabolic body size (EMBS) (fat-free mass + 17.7 kg) as described (7,36).

Intravenous glucose tolerance test. Early-phase insulin secretion was measured in response to a 25-g intravenous glucose bolus with calculation of the acute insulin response (AIR) as the average incremental plasma insulin concentration from the third to the fifth minute after the glucose bolus (7,36).

Analytic procedures. Plasma glucose concentrations were determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA). Plasma insulin concentrations were measured by radioimmunoassay, using either the Herbert modification of the method of Yalow and Berson or an automated analyzer (Concept 4; ICN, Costa Mesa, CA) (7,36). The mean fasting plasma glucose and insulin concentration was calculated as the average of three fasting plasma glucose and insulin concentrations assessed on separate days during the same admission.

Statistical analysis. Statistical analysis was performed using the procedures of the SAS Institute (Cary, NC). Results are given as means ± SD.

First, we analyzed the baseline data from all 384 subjects to examine the relationship between the fasting plasma insulin concentration and insulin sensitivity in Pima Indians and Caucasians. Stepwise and general linear regression models were used to identify determinants of the fasting plasma insulin concentration, to assess the variance (R^2) explained by these factors, and to generate the residuals of the fasting plasma insulin concentration after adjusting for its determinants.

Then, using data from 262 of the 319 Pima Indians who had follow-up OGTTs, we examined prospectively whether a high fasting plasma insulin concentration predicted diabetes independent of insulin resistance. At follow-up, 48 subjects had developed diabetes over an average of 7.1 ± 3.3 years (progressors), whereas the other 214 still had NGT or had developed IGT over a comparable period (6.2 ± 3.9 years, nonprogressors) (Table 1). Risk factors for diabetes were estimated by proportional hazards analysis (7). The predictive effects of the fasting plasma insulin concentration, M, and AIR (all log-transformed to achieve a normal distribution) were evaluated by entering these into the model as continuous variables. For presentation, the effects of these variables were expressed as relative hazards and were evaluated at the 10th and 90th percentiles of the predictor variables, with additional adjustment for age, sex, and percent body fat. Accordingly, the relative hazard estimates the risk of developing diabetes of a hypothetical subject at the 90th percentile compared with the risk of a hypothetical subject at the 10th percentile. For each relative hazard, the 95% CI is given (Table 2). The relationship between the fasting plasma insulin concentration and diabetes risk was also examined by computing the cumulative incidence (Kaplan-Meier method) among tertile groups defined by the residual fasting plasma insulin concentration.

In a third analysis, we used data from 161 of the 214 subjects who had not developed diabetes and had follow-up measurements of insulin sensitivity and early-phase insulin secretion (mean follow-up duration 5.1 ± 3.9 years) (Table 1). The aim of this analysis was to test whether the degree of relative insulinemia at baseline would predict subsequent changes in early-phase insulin secretion and/or insulin sensitivity before the onset of diabetes. Differences (Δ) in AIR (adjusted for age, sex, percent body fat, and M) and M (adjusted for age, sex, and percent body fat) were used as measures for the change in early-phase insulin secretion and insulin sensitivity, respectively.

Finally, we analyzed the baseline data of the 319 Pima Indians (representing 192 nuclear families) to assess the familiality of the residual fasting plasma insulin concentration. The range of the mean family-adjusted residual fasting plasma insulin concentration and the mean range within families were calculated as described (38). A genome-wide autosomal scan was then undertaken to identify loci linked to the residual fasting plasma insulin concentration. Subjects were genotyped at 516 polymorphic microsatellite markers distributed on all 22 autosomes with a median distance between markers of 6.4 cM (range 0.1–25.6) as previously described (39). Multipoint linkage analyses were performed using a variance components method (40).

RESULTS

The anthropometric and metabolic characteristics of the subjects in the different analyses are shown in Table 1.

Determination of relative hyper- and hypoinsulinemia. There was a significant inverse relationship between the fasting plasma insulin concentration and M in both Pima Indians

TABLE 1
Physical and metabolic characteristics of the subjects in the different analyses

	Baseline population		Prospective analysis of diabetes (n = 262)		Prospective analysis of AIR and M (n = 161)	
	Pimas	Caucasians	Nonprogressors	Progressors	Baseline	Follow-up
n	319	65	214	48	—	—
F/M	113/206	29/36	72/142	23/25	56/105	
Age (years)	26.2 ± 5.9	28.9 ± 7.5	26.4 ± 6.1	26.6 ± 6.0	25.6 ± 5.5	30.7 ± 6.2*
Height (cm)	167 ± 8	171 ± 9	167 ± 8	166 ± 8	167 ± 7	167 ± 7
Body weight (kg)	92.6 ± 22.8	92.9 ± 24.1	91.1 ± 22.7	98.9 ± 19.4†	93.9 ± 21.6	102.2 ± 25.2*
Body fat (%)	31 ± 9	29 ± 11	31 ± 9	35 ± 7‡	32 ± 9	34 ± 8*
Fat mass (kg)	30.0 ± 13.3	28.3 ± 15.1	29.0 ± 13.1	35.0 ± 11.9†	31.1 ± 13.7	35.8 ± 15.1*
Fat-free mass (kg)	62.6 ± 12.8	64.6 ± 13.3	62.1 ± 12.6	63.9 ± 11.3†	62.8 ± 11.6	66.4 ± 13.2*
Waist-to-thigh ratio	1.63 ± 0.15	1.52 ± 0.14	1.62 ± 0.15	1.65 ± 0.16‡	1.62 ± 0.15	1.70 ± 0.16*
Fasting plasma glucose (mg/dl)	88 ± 9	88 ± 9	88 ± 9	95 ± 7*	92 ± 7	92 ± 8
2-h Glucose (mg/dl)	110 ± 20	106 ± 18	110 ± 18	121 ± 13*	112 ± 18	128 ± 27*
Fasting plasma insulin (μU/ml)	36 ± 18	23 ± 9	35 ± 18	43 ± 20‡	35 ± 17	44 ± 21*
2-h Insulin (μU/ml)	148 ± 107	100 ± 98	138 ± 22	196 ± 124*	149 ± 107	242 ± 204*
M (mg · kg^{-1} EMBS · min^{-1})	2.8 ± 1.1	3.8 ± 1.5	2.9 ± 1.2	2.3 ± 0.6*	2.7 ± 0.9	2.4 ± 0.8*
AIR (μU/ml)§	257 ± 164	135 ± 82	267 ± 176	205 ± 137‡	265 ± 177	267 ± 186

Data are means ± SD. Symbols indicate significant differences between nonprogressors and progressors at baseline (prospective analysis of diabetes) or significant changes over time (prospective analysis of changes in AIR and M) (*P < 0.001, †P < 0.05, ‡P < 0.01). §AIR was assessed in 301 of the 319 Pima Indians in the baseline population, in all 262 subjects in the prospective analysis of diabetes, and in 131 of the 161 subjects in the prospective analysis of changes in AIR and M. To convert values for glucose to millimoles per liter, multiply by 0.056, and to convert values for insulin to picomoles per liter, multiply by 6.

and Caucasians, i.e., fasting plasma insulinemia increased with increasing insulin resistance and vice versa (Fig. 1). However, at any given M, there was considerable interindividual variability in the fasting plasma insulin concentration, with some individuals being relatively hyperinsulinemic for their degree of insulin sensitivity (above the regression line) and others being relatively hypoinsulinemic (below the regression line) (Fig. 1). In a stepwise multiple regression analysis, M was the single most important determinant of the fasting plasma insulin concentration, explaining 55% of its variability (R^2 = 0.55). Age, percent body fat, and waist-to-thigh ratio were significant additional determinants, explaining another 12% (i.e., a total of 67%) of the variance in the fasting plasma insulin concentration. After adjustment for the above covariates (age, sex, percent body fat, waist-to-thigh ratio, and M), subjects were classified based on their residual fasting plasma insulin concentration into those that are relatively hyperinsulinemic (positive residual) and those that are relatively hypoinsulinemic (negative residual). The residual fasting plasma insulin concentration was higher in Pima Indians than in Caucasians (P < 0.01) and was negatively correlated with the fasting plasma glucose concentration in both ethnic groups (Fig. 1).

Does relative hyperinsulinemia predict diabetes? In a proportional hazards analysis with adjustment for age, sex, and percent body fat, a high fasting plasma insulin concentration was a significant independent predictor of diabetes in Pima Indians with NGT in addition to low M and low AIR (Table 2). The two latter variables, previously shown to be risk factors for diabetes in this population (7), remained significant predictors after inclusion of the fasting plasma insulin concentration (Table 2) (i.e., even after accounting for adiposity, insulin sensitivity, and early-phase insulin secretion, a high fasting plasma insulin concentration predicted diabetes). Accordingly, after adjustment for age, sex, percent body fat, M, and AIR, individuals with a fasting plasma insulin concentration at the upper 90th percentile had a sixfold higher risk of developing diabetes than individuals with a fasting plasma insulin concentration at the lower 10th percentile (Table 2). The Kaplan-Meier curve for the 7-year cumulative incidence of diabetes indicates that the independent effect of hyperinsulinemia on diabetes risk increased progressively with increasing follow-up duration (Fig. 2). The fasting plasma glucose concentration was not a significant additional predictor of diabetes when entered in the above model (P = 0.79).

Does relative hyperinsulinemia predict a decline in insulin secretion and/or insulin sensitivity before the onset of diabetes? There was a negative correlation between the residual fasting plasma insulin concentration at baseline and the subsequent change (Δ) in AIR (Fig. 3), indicating that individuals who were relatively hyperinsulinemic (positive residual) at baseline were more predisposed to a decline in early-phase insulin secretion than those who were relatively hypoinsulinemic at baseline. This was confirmed by the results of a Cox proportional hazards analysis, which showed that an individual with a residual fasting plasma insulin concentration at the 90th percentile had a twofold higher risk of a decrease in AIR than a person at the 10th percentile, although, in that analysis, the trend only approached statistical significance (P = 0.07). There was no relationship between the residual fasting plasma insulin concentration at baseline and the subsequent change in insulin sensitivity (ΔM) (Fig. 3).

Do familial/genetic factors determine relative hyperinsulinemia? Among the 319 Pima Indians with NGT, family membership was a significant determinant of the fasting plasma insulin concentration, independent of age, sex, percent body fat, waist-to-thigh ratio, and M. The family effect is illustrated in Fig. 4, demonstrating that the mean residual fasting plasma insulin concentration was approximately three times more variable between families than within families. The heritability (h^2) of the residual fasting plasma

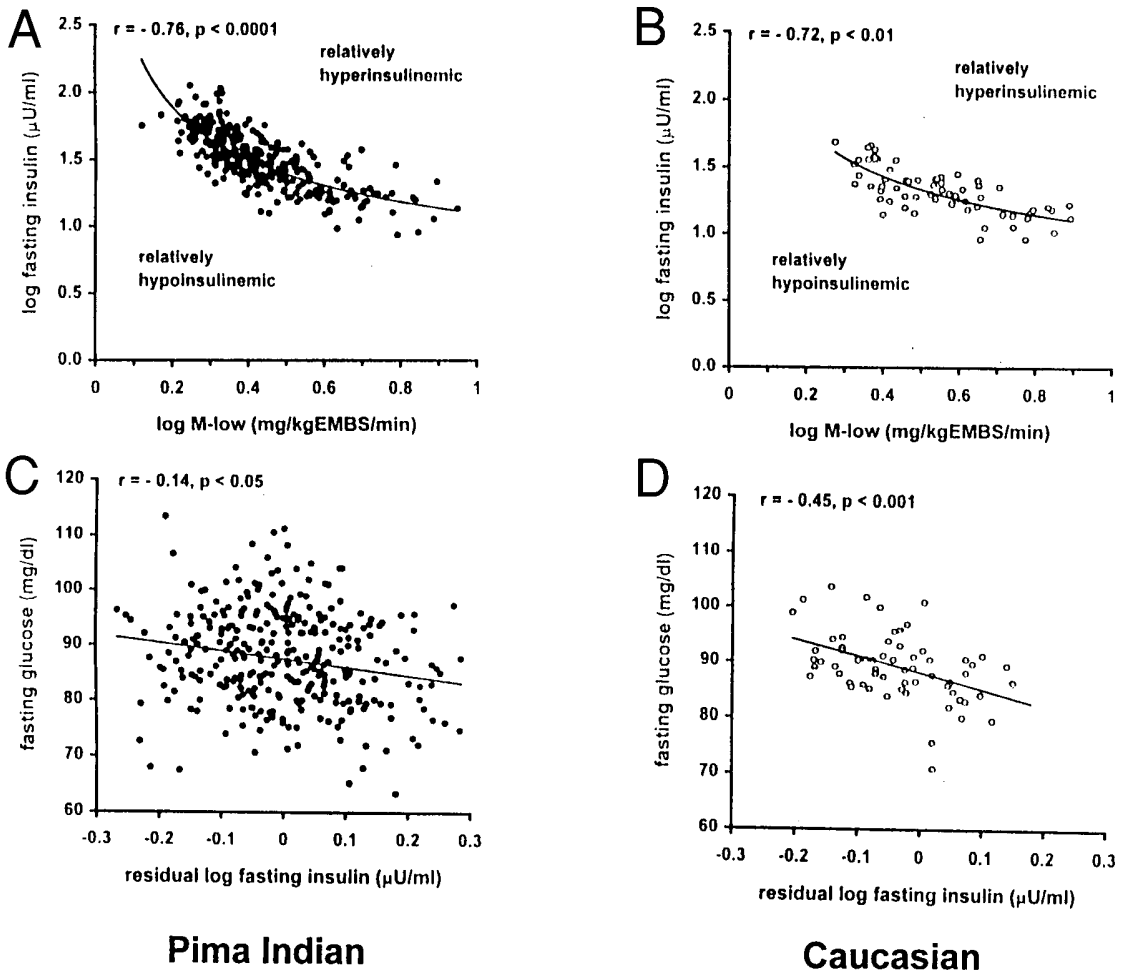

FIG. 1. Relationships between the fasting plasma insulin concentration and insulin sensitivity (*M*) (*A* and *B*) and between the fasting plasma glucose concentration and the residual fasting plasma insulin concentration (adjusted for age, sex, percent body fat, waist-to-thigh ratio, and *M*) (*C* and *D*) in 319 Pima Indians (*A* and *C*) and 65 Caucasians (*B* and *D*) with NGT (individual data and regression line).

insulin concentration, estimated from the variances in the above model, was 0.52 ($P < 0.01$). In the genomic scan, only one chromosomal region showed possible evidence for linkage (logarithm of odds [LOD] score >1.5) with the residual fasting plasma insulin concentration. The peak of this linkage (LOD score 1.77) was located at a genetic distance of 188 cM on the long arm of chromosome 3 (3q26.1). Among the genes located within the support interval (LOD score –1) of the identified region (169–201 cM) is the gene for the glucose transporter 2 (*GLUT2*, also known as solute carrier family 2, member 2 [SLC2A2]) (Fig. 4). The linkage results of the entire genomic scan (including the remaining 21 autosomes) can be found in an online appendix at www.diabetes.org/diabetes/appendix.asp.

DISCUSSION

Fasting hyperinsulinemia is common in individuals with impaired glucose homeostasis (1–4), predicts the development of diabetes in various populations (11–22), and is a widely accepted surrogate measure of insulin resistance. In the present series of studies, we aimed to test the hypothesis (26,27,34,35) that fasting hyperinsulinemia itself may have a primary pathogenic role in the development of diabetes, independent of insulin resistance.

The results indicate that among Pima Indians with NGT, a high fasting plasma insulin concentration is an independent risk factor for diabetes, in addition to insulin resistance and impaired early-phase insulin secretion. Further analyses revealed that individuals with a high relative fasting plasma insulin concentration (for their degree of adiposity and insulin resistance) are at increased risk for a decline in early-phase insulin secretion, but not in insulin sensitivity, before the onset of diabetes. Finally, we demonstrate that the adjusted fasting plasma insulin concentration is a familial trait possibly linked to a locus on chromosome 3q, which harbors the gene for *GLUT2*. Together, these findings provide the first prospective evidence in humans to support the hypothesis (26,27,34,35) that fasting hyperinsulinemia, possibly reflecting primary hypersecretion of insulin, plays an independent pathogenic role in the development of diabetes and is not simply a surrogate measure of insulin resistance.

The results of our baseline analysis confirm the well-established finding (23) that fasting plasma insulin concentration and insulin sensitivity are inversely related, i.e., that cross-sectionally, fasting insulinemia increases with increasing insulin resistance and vice versa. However, our results also indicate that only ~50% of the variability in the fasting plasma insulin concentration can be explained by insulin sensitivity. At any

TABLE 2
Multivariate proportional hazards model of independent predictors of type 2 diabetes in 262 Pima Indians with NGT followed for 6.4 ± 3.9 years

	Value at 10th percentile	Value at 90th percentile*	Relative hazard†	95% CI	*P*
M (mg · kg^{-1} EMBS · min^{-1})	1.8	4.3	5.8	1.1–31.3	<0.04
AIR (μU/ml)	95	470	9.6	4.1–22.7	<0.0001
Fasting plasma insulin concentration (μU/ml)	17	60	6.0	1.2–29.5	<0.03

This model is adjusted for age, sex, and percent body fat. To convert values for glucose to millimoles per liter, multiply by 0.056, and to convert values for insulin to picomoles per liter, multiply by 6. *For *M* and AIR, the value at the 90th percentile is the value associated with the lower risk for diabetes, whereas for the fasting plasma insulin concentration, the value at the 10th percentile is associated with the lower risk. †Hazard rate is for a hypothetical subject at the percentile with the higher risk of diabetes divided by the hazard rate for a hypothetical subject at the percentile with the lower risk.

given degree of insulin sensitivity, there was substantial interindividual variability in the fasting plasma insulin concentration, with some individuals being relatively hyperinsulinemic and others relatively hypoinsulinemic for their degree of insulin sensitivity. If hyperinsulinemia was solely a secondary phenomenon to compensate for insulin resistance, then one might expect that individuals with relative hyperinsulinemia would have a lower risk of diabetes than those who are relatively hypoinsulinemic.

When we followed the Pima Indians from our baseline cohort prospectively, however, we found that the opposite was the case. After adjusting for age, sex, and adiposity, a high fasting plasma insulin concentration was a significant independent predictor of diabetes, in addition to insulin resistance and low early-phase insulin secretion (i.e., individuals who were relatively hyperinsulinemic at baseline when they had NGT had a higher risk of developing diabetes than those who were relatively hypoinsulinemic). This finding represents an important extension of previous prospective findings that fasting hyperinsulinemia predicts diabetes (11–22). In those studies, insulin sensitivity was not directly quantified, but instead, fasting hyperinsulinemia was used as a surrogate marker of insulin resistance. In contrast, in the present study, a high fasting plasma insulin concentration predicted diabetes independent of a low rate of insulin-stimulated glucose disposal—a direct measure of insulin resistance. In the present study, therefore, a high fasting plasma insulin concentration is not a reflection of insulin resistance but may rather reflect a basal hypersecretion of insulin relative to the degree of insulin resistance. This result was further supported by our finding that in both Pima Indians and Cau-

FIG. 2. Kaplan-Meier curve for the 7-year cumulative incidence of type 2 diabetes in Pima Indians with NGT with a fasting plasma insulin concentration in the upper and lower tertile, after adjustment for age, sex, percent body fat, insulin sensitivity (*M*), and early-phase insulin secretion (AIR to glucose).

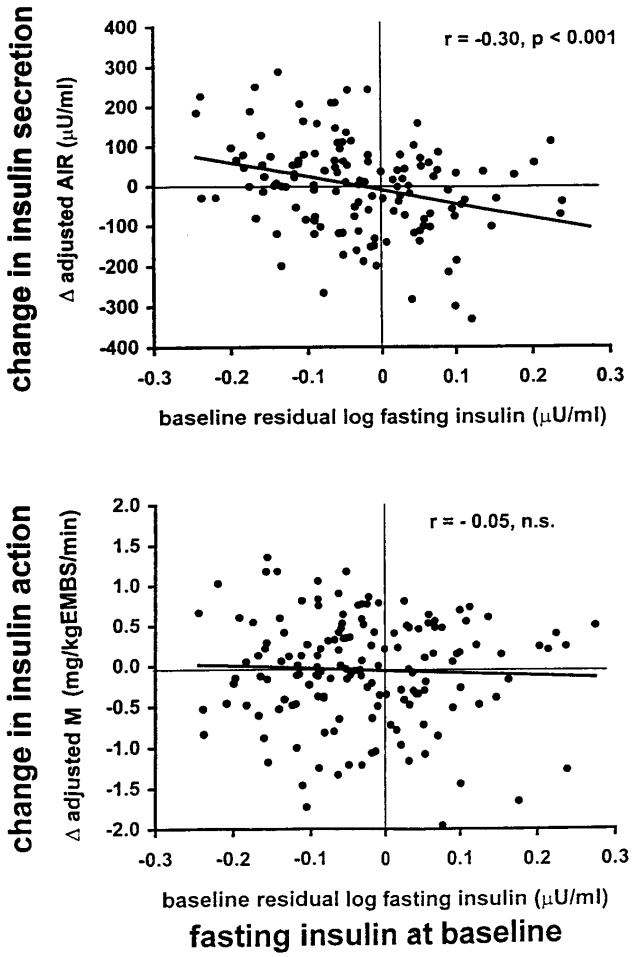

FIG. 3. Relationship between the residual fasting plasma insulin concentration at baseline (adjusted for age, sex, percent body fat, waist-to-thigh ratio, and *M*) and subsequent changes in early-phase insulin secretion (AIR to glucose, *n* = 131) and insulin sensitivity (*M*, *n* = 161) over 5.1 ± 3.9 years. EMBS = fat-free mass + 17.7 kg.

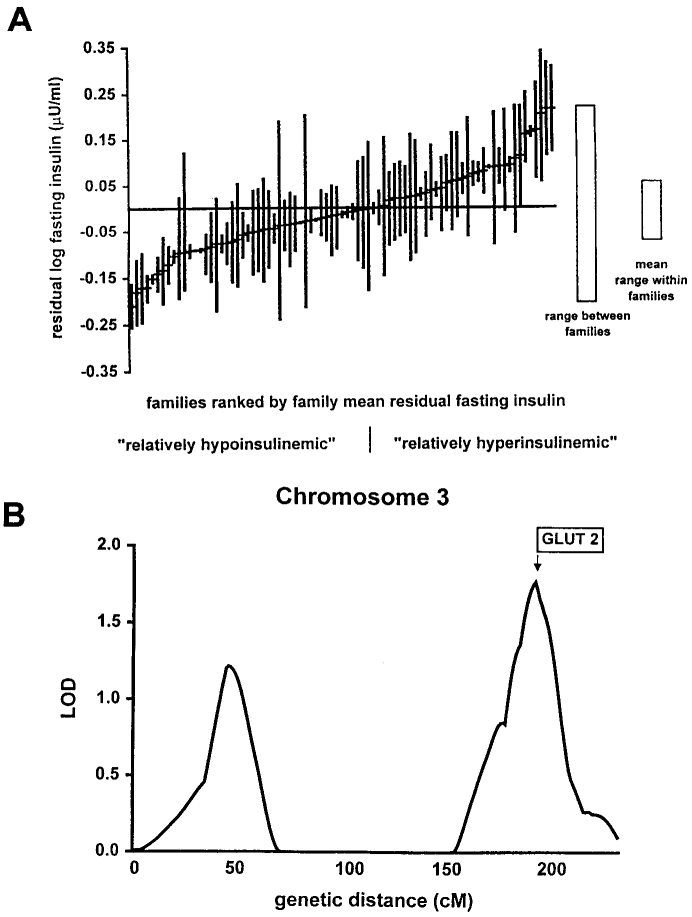

FIG. 4. ***A*****: Residual of the fasting plasma insulin concentration (adjusted for age, sex, percent body fat, waist-to-thigh ratio, and** ***M*****) ranked by family means. Each column represents one family and depicts the mean, minimum, and maximum value for a family. Note that there is a much larger variation among families than within families.** ***B*****: Variance components linkage analysis LOD score for the residual fasting plasma insulin concentration (adjusted for age, sex, percent body fat, waist-to-thigh ratio, and** ***M*****) plotted against the genetic distance on chromosome 3 (peak of linkage and support interval [1 – LOD score]: 188 [169–201] cM).**

casians, the residual fasting plasma insulin concentration was negatively related to the fasting plasma glucose concentration and not positively, as is the case with the unadjusted fasting plasma insulin concentration. This finding clearly indicates that relative hyperinsulinemia has physiological significance and is not simply an artifact of methodological limitations, such as in the quantification of insulin sensitivity with the hyperinsulinemic clamp technique or in the specificity of the insulin assay (cross-reactivity with proinsulin and split products). The hypothesis that fasting hyperinsulinemia itself may have a primary pathogenic role in the development of type 2 diabetes independent of insulin resistance has so far been based on indirect evidence such as the findings that experimental hyperinsulinemia causes insulin resistance in humans (25), that basal hypersecretion of insulin appears to be more common than insulin resistance in individuals with obesity (26), and that ethnic groups with high propensity for diabetes are markedly hyperinsulinemic (6,7,15,16,32). The present finding that a high fasting plasma insulin concentration predicts type 2 diabetes independent of insulin resistance is the first prospective evidence in humans to support the concept of an independent pathogenic role of primary hyperinsulinemia.

Having established that relative hyperinsulinemia predisposes individuals with NGT to diabetes, we then aimed to identify possible mechanisms by which this could occur. Because experimental studies suggest that chronic hyperinsulinemia may have detrimental effects on both early-phase insulin secretion (35,41) and insulin sensitivity (24,25), we examined the relationship between the residual fasting plasma insulin concentration at baseline and subsequent changes in these two measures. We found that NGT individuals who were relatively hyperinsulinemic at baseline were more likely to experience a decline in AIR, but not in *M*, compared with those with relative hypoinsulinemia. This finding suggests that the independent effect of fasting hyperinsulinemia to predict diabetes may at least in part be mediated by a detrimental effect on early-phase insulin secretion. One possible explanation for this is that chronic hypersecretion of insulin reduces the amount of insulin available for immediate release. An interesting alternative explanation has recently emerged from the finding that tissue-specific knockout of the insulin receptor in pancreatic β-cells leads to abnormalities in insulin secretion, including impaired early-phase insulin secretion and fasting hyperinsulinemia (42). Based on these findings, it could be proposed that chronic hyperinsulinemia may result in a downregulation of insulin receptors in pancreatic β-cells (β-cell insulin resistance), leading to impaired glucose sensing and thereby impaired early-phase insulin secretion. Because some (43,44) but not all (45) studies suggest that hyperinsulinemia may be a risk factor for body weight gain and because weight gain in turn is a well-established risk factor for diabetes (46), it was also possible that the independent effect of hyperinsulinemia to predict diabetes was mediated by an effect on body weight. This possibility was unlikely, however, because separate prospective analyses showed that relative hyperinsulinemia is not a predictor of weight gain in Pima Indians (data not shown).

The factors determining whether an individual with NGT is hyper- or hypoinsulinemic relative to the degree of insulin sensitivity and adiposity remain to be fully identified, but differences in β-cell size and mass (31), in plasma free fatty acid concentration and/or tissue (islet and muscle) triglyceride or glycogen content (28), in β-cell insulin receptor expression (42), in the constitutive secretory pathway (31), in the metabolic clearance rate of insulin (32), in central (hypothalamic) regulatory pathways (27,30), and/or in the activity of the parasympathetic nervous system (27,29,30) have been suggested. The latter mechanism is of particular interest because recent evidence suggests that the marked hyperinsulinemia in Pima Indians may, at least in part, be due to an increased vagal cholinergic drive to the pancreas (34). Finally, our demonstration that relative hyperinsulinemia is a highly heritable trait aggregating in families strongly suggests that genetic factors are involved in determining whether a person is relatively hyper- or hypoinsulinemic. In an attempt to identify possible chromosomal loci linked to the adjusted fasting plasma insulin concentration, we undertook a genome-wide autosomal scan. The only region with suggested evidence for linkage was on the long arm of chromosome 3. Although the strength of the linkage signal (LOD

score = 1.77) is not sufficient for this to be considered significant evidence for linkage (which typically requires a LOD score >3.0), the identified region is interesting because the peak of the linkage coincided quite precisely with the gene for *GLUT2* (47). *GLUT2* is the glucose transporter isoform expressed in pancreatic β-cells that plays an important role in the regulation of glucose-stimulated insulin secretion (48,49) and has previously been suggested as a candidate gene for type 2 diabetes (49). This is supported by findings that a mutation in *GLUT2* in a patient with diabetes abolished glucose transport activity (50), that *GLUT2* knockout mice develop early-onset diabetes (51), and that *GLUT2* expression is markedly reduced in glucose-unresponsive islets from animal models of type 2 diabetes (51). In a previous study in Pima Indians, we found that ~5% of this population carries a missense polymorphism in exon 3 of the *GLUT2* gene (52), but this polymorphism was not associated with the residual fasting plasma insulin concentration in the present study. Despite the fact that *GLUT2* is an attractive candidate, it remains possible that other, perhaps yet unknown, genes in this rather broad (~30 cM) region of linkage may account for the observed results.

The finding that relative hyperinsulinemia predicts diabetes in individuals with NGT may have important clinical implications. First, it may provide one possible explanation for the high prevalence of diabetes in ethnic groups with marked hyperinsulinemia, such as Native-Americans (7,21,22), Mexican-Americans (6,15), and Pacific Islanders (16). Second, our finding may explain, in part, individual and familial differences in diabetes risk and is therefore also likely to be relevant to populations with a lower prevalence of type 2 diabetes. Finally, from a preventative perspective, our results suggest that attenuation of hyperinsulinemia might be an option in the primary prevention of type 2 diabetes, just as restoration of insulin sensitivity and early-phase insulin secretion may delay or prevent deterioration of glucose tolerance. Pharmacological attenuation of insulin hypersecretion, such as with diazoxide, has previously been reported to improve (53) and even prevent (54) glucose intolerance in rodent models of obesity and type 2 diabetes and, more recently, to exert beneficial metabolic effects also in obese humans (55). In this respect, it is important to point out that our findings were exclusively obtained in individuals with NGT, not IGT, at baseline. Intervention studies are required to assess whether diabetes can be prevented by attenuation of hyperinsulinemia.

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