Increased Proinsulin Levels and Decreased Acute Insulin Response Independently Predict the Incidence of Type 2 Diabetes in the Insulin Resistance Atherosclerosis Study

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Previous studies have indicated that β-cell dysfunction predicts the development of diabetes, although it is unknown whether the use of combinations of insulin secretory measures further improves prediction. The Insulin Resistance Atherosclerosis Study is a prospective, multicenter, epidemiological study of the relationship between insulin sensitivity and the risk of diabetes and cardiovascular disease. At baseline, fasting concentrations of insulin, intact proinsulin (PI), and split PI were measured, and acute insulin response (AIR) was determined during a frequently sampled intravenous glucose tolerance test (FSIGTT). Subjects who were nondiabetic at baseline (n = 903) were reexamined after 5 years of follow-up; 148 had developed diabetes. In separate logistic regression models adjusted for age, sex, clinic, and ethnicity, 1 SD differences in measures of β-cell dysfunction were associated with diabetes incidence (AIR: odds ratio [OR] 0.37, 95% CI 0.27–0.52; intact PI: OR 1.90, 95% CI 1.57–2.30; split PI: OR 1.94, 95% CI 1.63–2.31). After additional adjustment for BMI, impaired glucose tolerance, and insulin sensitivity, these measures continued to be significantly associated with risk of diabetes (all $P < 0.0001$). Furthermore, in models that included both PI and AIR, each was an independent predictor, and individuals who had combined low AIR and high PI experienced the highest diabetes risk. In conclusion, both low AIR and high PI independently predicted diabetes in a well-characterized multiethnic population. Although fasting PI is simpler to assess, determining AIR from an FSIGTT may further improve prediction. If pharmacological agents to prevent diabetes are proved to be efficacious in ongoing clinical trials, then it may be beneficial to perform FSIGTTs to identify better (for intensive intervention) prediabetic subjects who would ultimately require lifelong pharmacological therapy. *Diabetes* 51: 1263–1270, 2002

β-Cell dysfunction is a key feature of the natural history of type 2 diabetes (1,2). A number of direct and surrogate measures are available for the documentation of β-cell dysfunction, including indexes derived from the hyperglycemic clamp technique, frequently sampled intravenous glucose tolerance test (FSIGTT), and the oral glucose tolerance test (OGTT) (3–5). Among the more detailed of these measures is the acute insulin response (AIR) during an FSIGTT (5), which captures the β-cell compensation to a challenge of glucose or arginine within the first 8–10 min of the FSIGTT (the first-phase insulin response). Decreased AIR has been documented in subjects with diabetes and impaired glucose tolerance (IGT), as well as among first-degree relatives of individuals with type 2 diabetes (2). Furthermore, decreased AIR has been associated prospectively with risk of developing diabetes in clinical and population-based studies (6–8). The risk of progression to diabetes associated with low insulin secretion is especially pronounced when the degree of background insulin resistance is also considered, a phenomenon that has been demonstrated in both prospective observational (6,7,9) and detailed cross-sectional studies (10). This observation highlights the increasing departure, among prediabetic individuals, from the hyperbolic balance between insulin sensitivity and insulin response that exists in the healthy state (10).

The increasing availability of assays for the measurement of proinsulin (PI) concentration has also contributed to the understanding of the natural history of β-cell dysfunction (11). PI and its split products circulate in high concentrations in subjects with diabetes and gestational diabetes (12–14), as well as (in some studies) among individuals with IGT or a diabetic first-degree relative (12,15,16). In addition, a limited number of investigations have reported that elevated absolute and relative concentrations of PI and its split products are prospectively

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AIR, acute insulin response; FFA, free fatty acid; FSIGTT, frequently sampled intravenous glucose tolerance test; IGT, impaired glucose tolerance; IRAS, Insulin Resistance Atherosclerosis Study; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; OR, odds ratio; PI, proinsulin; S_I, insulin sensitivity index.

associated with risk of diabetes (17–23). Finally, recent studies have shown that PI and the PI-to-insulin ratio correlate significantly with AIR (24,25). This body of literature provides support for the hypothesis that PI can be used as a sensitive marker of early β-cell dysfunction (11).

There are, however, a number of gaps in the literature regarding insulin secretory dysfunction and risk of diabetes. First, in the majority of studies, only surrogate indexes of insulin resistance have been used as covariates, and these measures may not adequately account for background levels of insulin resistance when considering the causative role of insulin secretion. Second, there have been no studies of PI and glycemic progression among black or Hispanic individuals, and few studies have had adequate numbers of subjects with IGT to consider the importance of PI as a predictor in this subgroup. Finally, it is unknown whether the use of combinations of different insulin secretory measures further improves prediction, a construct that may have etiologic and public health implications.

The objectives of this article were to examine the association of baseline levels of intact and split PI and AIR with the 5-year risk of diabetes using data from the Insulin Resistance Atherosclerosis Study (IRAS), which is following a large, multiethnic, multicenter cohort of middle-aged individuals at various stages of glucose intolerance. In addition, we wanted to determine whether the use of combinations of insulin secretory measures, including high PI (or split PI) and low AIR, further improves prediction of diabetes development.

RESEARCH DESIGN AND METHODS

Study subjects. The IRAS is a multicenter, observational, epidemiological study of the relationships between insulin resistance and cardiovascular disease and its known risk factors in different ethnic groups at varying states of glucose tolerance. The design and methods of this study have been described in detail in previous publications (26,27). Briefly, the study was conducted at four clinical centers. At centers in Oakland and Los Angeles, California, non-Hispanic white and black individuals were recruited from Kaiser Permanente, a nonprofit health maintenance organization. Centers in San Antonio, Texas, and San Luis Valley, Colorado, recruited white and Hispanic individuals from two ongoing population-based studies (the San Antonio Heart Study and the San Luis Valley Diabetes Study) (26). A total of 1,625 individuals participated in the baseline IRAS examination (56% women), which occurred between October 1992 and April 1994. The IRAS protocol was approved by local institutional review committees, and all participants provided written informed consent.

After an average of 5.2 years (range, 4.5–6.6 years), follow-up examinations of this cohort were conducted using the protocol used at baseline. The response rate was 81%, and those who attended the follow-up examination were similar to those who did not attend in terms of ethnicity, sex, baseline glucose tolerance status (normal glucose tolerance [NGT] versus IGT), and BMI (all comparisons, $P > 0.32$). The present article includes information on 906 individuals who were free of diabetes at baseline and for whom information was available on intact and split PI, insulin, insulin sensitivity index (S_I), AIR, and covariates.

Clinical measurements and procedures. The IRAS protocol required two visits, 1 week apart, of ~4 h duration each. Subjects were asked before each visit to fast for 12 h, to abstain from heavy exercise and alcohol for 24 h, and to refrain from smoking on the morning of the examination. During the first visit, a 75-g OGTT was administered, with glucose tolerance status determined using World Health Organization Criteria (28). During the second visit, insulin sensitivity and insulin secretion were determined using an FSIGTT, with two modifications to the original protocol (29). First, an injection of regular insulin, rather than tolbutamide, was used to ensure adequate plasma insulin levels for the accurate computation of insulin sensitivity across a broad range of glucose tolerance (30). Second, a reduced sampling protocol (with 12 rather than 30 samples) was used for efficiency, given the large number of participants (31). Insulin sensitivity, expressed as the S_I, was calculated using mathematical modeling methods (MINMOD version 3.0) (32). First-phase insulin secretion, expressed as the AIR, was defined as the mean increment in the plasma insulin concentration above basal in the first 8 min after the administration of glucose. The repeatability of both S_I and AIR have been demonstrated in a subsample of the IRAS cohort (33). The estimate of S_I from this modified protocol has been validated against gold standard measures of insulin resistance from the hyperinsulinemic-euglycemic clamp technique (34). AIR has been validated by others using gold standard measures of insulin secretion from the hyperglycemic clamp technique (5).

Height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively. BMI was calculated as weight/height^2 (kg/m^2) and was used as an estimate of overall adiposity. Waist circumference, a validated estimate of visceral adiposity (35), was measured to the nearest 0.5 cm using a steel tape. Duplicate measures were made following a standardized protocol, and averages were used in the analysis. Ethnicity was assessed by self-report.

Laboratory procedures. Glucose concentration was determined using standard methods as described previously (26). Insulin levels were measured using the dextran-charcoal radioimmunoassay (36), which has a 19% external coefficient of variation. This assay displays a high degree of cross-reactivity with PI. Fasting serum intact PI and 32-33 split PI were determined from samples that had been stored at −70°C for an average of 3.3 years using highly specific two-site monoclonal antibody–based immunoradiometric assays (37,38). These assays each have a sensitivity limit of 1.25 pmol/l and split pair coefficients of variation of 14% for PI ($n = 98$) and 18% for split PI ($n = 98$). The intact PI assay shows no detectable cross-reactivity with insulin or 32-33 split PI. The split PI assay shows no cross-reactivity with insulin, although it cross-reacts equally with 32-33, des-32, and des-31-32 split PIs, and thus the term 32-33 split PI is used here to indicate the sum of these three molecules, the majority of which are des-31-32 split PI. In addition, the split PI assay cross-reacts substantially (84%) with intact PI, and thus split PI assay values were corrected by subtraction of the corresponding PI cross-reactivity.

Statistical analyses. Means and SDs, or proportions, were presented for subjects stratified by follow-up diabetes status. Differences between groups were assessed using t tests or χ^2 tests, as appropriate (Table 1). Associations between baseline measures of β-cell function (AIR, insulin, and intact and split PI) and baseline anthropometric and metabolic variables were determined using Spearman correlation analysis, adjusted for age, sex, ethnicity, and clinical center.

Associations between β-cell function and risk of diabetes development were assessed using multiple logistic regression analysis. In these models, the dependent variable was incident diabetes at the follow-up visit, and independent variables included baseline measures of AIR, intact PI, split PI, and insulin. Given the potential statistical problems associated with ratio variables, including undesirable effects on error distributions and the possible introduction of spurious correlations (39,40), we examined intact and split PI as independent variables in models that also included fasting insulin as a covariate (i.e., after linear adjustment for fasting insulin). Odds ratios (ORs) and 95% CI were calculated per 1-SD increase in the independent variable at baseline. To investigate the possibility of nonlinear associations, we also modeled measures of insulin secretion using quartiles (Fig. 1). To determine whether ethnicity, baseline glucose tolerance status (NGT versus IGT), baseline visceral adiposity (<sex-specific median versus ≥sex-specific median waist circumference), or baseline insulin resistance (<median S_I versus ≥median S_I) modified the associations between measures of β-cell function and risk of diabetes, we included interaction terms in separate demographically adjusted models ($P < 0.05$ considered significant). In addition, we examined this issue by calculating the ORs and 95% CI for each strata of the interaction variable under consideration (Table 2). We examined the effect of confounding factors by fitting three models for each independent variable. Model A was adjusted for age, sex, ethnicity, and clinic (the demographic variables); model B was adjusted for demographic variables plus BMI and IGT; and model C was adjusted for demographic variables, BMI, IGT, and S_I (Table 3).

Finally, we assessed the combined effects of low AIR and high PI concentration by including AIR and intact (or split) PI in the same multivariate model. In addition, we analyzed risk of diabetes by four strata of increasing β-cell dysfunction. These strata were defined as *1*) high AIR and low intact PI, *2*) high AIR and high intact PI, *3*) low AIR and low intact PI, and *4*) low AIR and high intact PI. Similar analyses were conducted using split PI in place of intact PI. "Low" and "high" were defined by the median value of the stratifying variable (AIR = 374.41 pmol · ml^{-1} · min^{-1}; intact PI = 4.5 pmol/l; split PI = 5.7 pmol/l), and the associations were examined after adjustment for demographic variables, BMI, IGT, and S_I (model C) (Fig. 2).

TABLE 1
Baseline demographic, anthropometric, and metabolic characteristics of nondiabetic participants in the IRAS, according to conversion to type 2 diabetes at 5-year follow-up

Baseline variable	Nonconverters	Converters	*P* value*
n	758	148	
Sex (F/M)	56.07/43.93	60.14/39.86	0.36
Ethnicity (NHW, black, His)	40.50/26.25/33.25	37.16/27.70/35.14	0.75
Glucose tolerance (NGT/IGT)	73.35/26.65	31.76/68.24	<0.0001
Age (years)	54.37 ± 8.53	56.04 ± 7.78	0.03
BMI (kg/m^2)	27.89 ± 5.30	31.13 ± 6.34	<0.0001†
Waist circumference (cm)	89.25 ± 12.41	95.53 ± 13.06	<0.0001†
Fasting glucose (mg/dl)	96.91 ± 10.30	106.63 ± 11.96	<0.0001†
2-h glucose (mg/dl)	119.36 ± 31.51	152.77 ± 30.80	<0.0001
Fasting insulin (μU/ml)	14.36 ± 11.14	22.23 ± 25.72	<0.0001†
Fasting intact PI (pmol/l)	5.41 ± 4.72	9.33 ± 7.01	<0.0001†
Fasting split PI (pmol/l)	7.16 ± 6.81	13.68 ± 11.53	<0.0001†
Insulin sensitivity $S_1 \times 10^{-4}$ ($min^{-1} \cdot \mu U^{-1} \cdot ml^{-1}$)	2.37 ± 2.06	1.26 ± 1.56	<0.0001†
AIR ($pmol \cdot ml^{-1} \cdot min^{-1}$)	529.38 ± 506.48	275.46 ± 305.46	<0.0001

Data are % and means ± SD unless otherwise indicated; sample sizes vary slightly as a result of occasional missing values. His, Hispanic; NHW, non-Hispanic white. *P values derived from χ^2 or t tests, as appropriate; †Test performed on log-transformed variable.

RESULTS

Baseline characteristics of nondiabetic participants in the IRAS, stratified by diabetes status at the 5-year follow-up, are presented in Table 1. Individuals who had IGT at baseline were more likely to have converted to type 2 diabetes after 5 years ($P < 0.0001$). In addition, converters to type 2 diabetes were significantly older ($P = 0.03$), were more obese (in terms of both total and abdominal adiposity), and had higher concentrations of glucose and insulin and lower S_I compared with nonconverters (all $P < 0.0001$). Furthermore, concentrations of intact and split PI were significantly higher and AIR was significantly lower in converters compared with nonconverters (PI, split PI, and AIR, $P < 0.0001$). Stratified analyses by baseline glucose tolerance status revealed generally similar patterns in the NGT and IGT subgroups (data not shown).

Baseline AIR was not associated with intact PI ($r = 0.06$, NS) and was only modestly correlated with split PI ($r = 0.09$, $P = 0.01$), insulin ($r = 0.21$, $P < 0.0001$), S_I ($r = -0.19$, $P < 0.00001$), and BMI ($r = 0.13$, $P = 0.0002$). Intact and split PI, conversely, displayed moderate to strong associations with insulin ($r = 0.56$ and $r = 0.62$, respectively, both $P < 0.0001$), S_I ($r = -0.54$ and $r = -0.60$, both $P < 0.0001$), BMI ($r = 0.47$ and $r = 0.51$, both $P < 0.0001$), and fasting glucose ($r = 0.45$ and $r = 0.41$, both $P < 0.0001$).

In models adjusted for age, sex, ethnicity, and clinic, risk of diabetes decreased across quartiles of AIR and increased across quartiles of intact and split PI and insulin (Fig. 1) (all $P < 0.0001$ for trend), with only modest evidence of nonlinearity for split PI. The associations of 1-SD changes in these insulin secretory measures with risk of diabetes were next investigated within strata of potential effect modifying variables (Table 2), including ethnicity, baseline glucose tolerance status (NGT versus IGT), baseline visceral adiposity (<sex-specific median versus ≥sex-specific median waist circumference), and baseline insulin sensitivity (<median S_I versus ≥median S_I). Although these analyses revealed a limited number of statistically significant interaction terms, there was no substantial evidence of important and consistent effect modification (i.e., no changes in the direction of associations, and no consistent effect modification across all β-cell measures), and therefore subsequent results are presented with these subgroups pooled.

Separate logistic regression models, adjusted for age, sex, ethnicity, and clinic, were fit to examine the effect of 1-SD changes in insulin secretory measures on type 2 diabetes incidence. Increased AIR was associated with a reduced risk of diabetes development (OR 0.37, 95% CI 0.27–0.52), whereas increased concentrations of PI, split PI, and insulin were associated with increased diabetes risk (PI: OR 1.90, 95% CI 1.57–2.30; split PI: OR 1.94, 95% CI 1.63–2.31; insulin: OR 1.68, 95% CI 1.34–2.10). Although the areas under the receiver operator characteristic curves were larger for both the intact and split PI models compared with the insulin model, these differences were not statistically significant ($\chi^2 = 1.94$, $P = 0.17$; $\chi^2 = 2.21$, $P = 0.14$; respectively). In addition, there was very little difference in the areas under the receiver operator characteristic curves between the intact and split PI models ($\chi^2 = 0.002$, $P = 0.96$). Intact and split PI continued to be significant predictors of diabetes after linear adjustment for fasting insulin (PI: OR 1.74, 95% CI 1.43–2.21; split PI: OR 1.79, 95% CI 1.49–2.15). Increased S_I was associated with a reduced risk of diabetes development (OR 0.32, 95% CI 0.23–0.47). With additional adjustment for BMI and IGT, all insulin secretory measures maintained their significance as diabetes predictors, although the ORs were attenuated in each case (data not shown). When insulin sensitivity was further taken into account, the magnitude of the OR associated with AIR increased (OR 0.32, 95% CI 0.22–0.47), as would have been predicted given the hyperbolic relationship between insulin resistance and insulin secretion (10). ORs associated with intact PI, split PI, and insulin were only slightly reduced with adjustment for S_I (data not shown). Other significant predictors of diabetes in fully adjusted models included IGT (all models), S_I (all models except split PI), and BMI (AIR model only) (data not shown). When AIR, intact and split PI, and insulin were included in the same fully adjusted model, only decreased

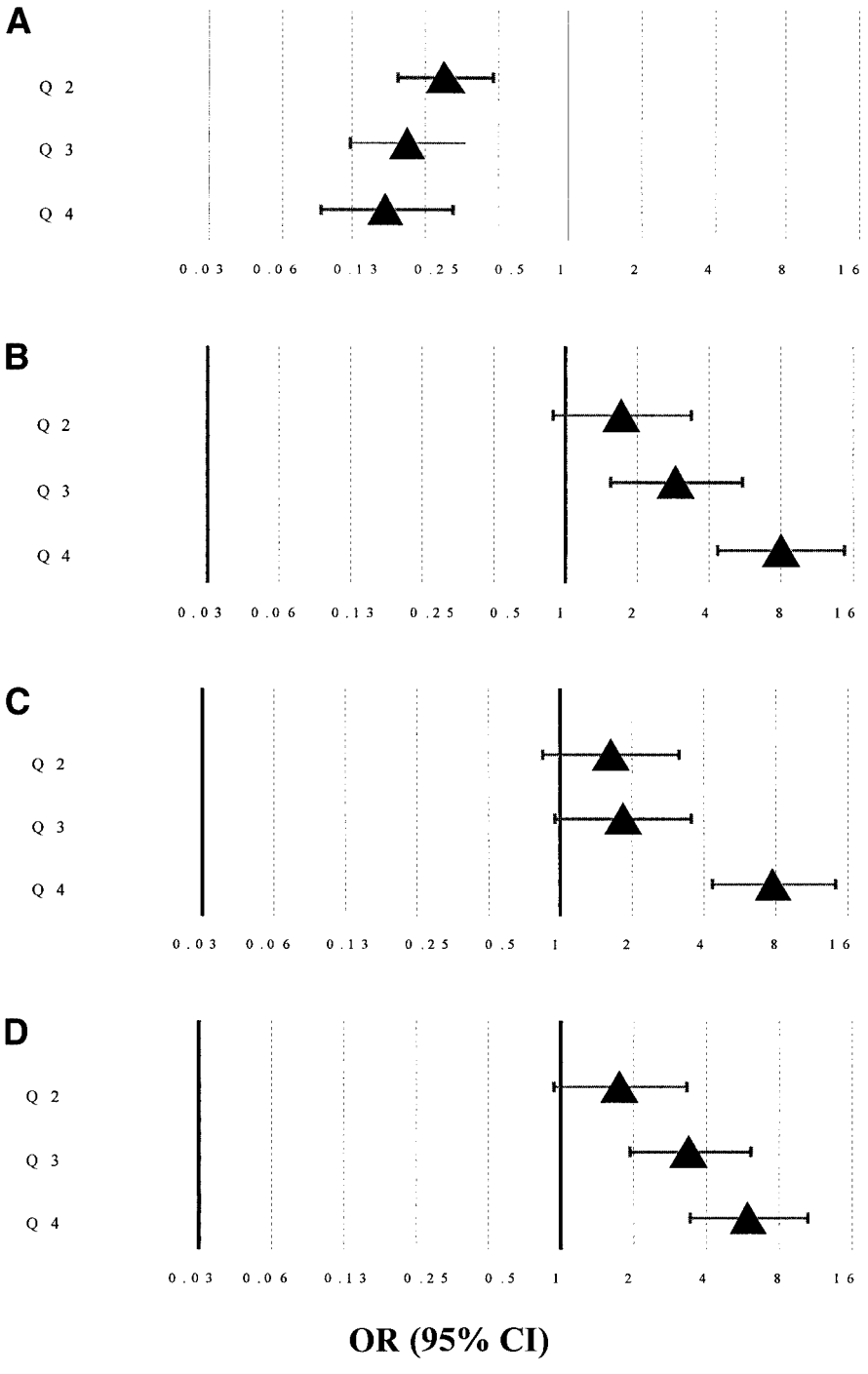

FIG. 1. Multiple logistic regression analysis of measures of β-cell function and 5-year risk of type 2 diabetes in IRAS, adjusted for age, sex, ethnicity, and clinic. ORs are presented for the second, third, and fourth quartiles of each independent variable (all $P < 0.0001$ for trend). *A*: AIR, quartile cut points: 161.5, 374.4, and 667.4. *B*: Intact PI, quartile cut points: 3.1, 4.5, and 7.2. *C*: Split PI, quartile cut points: 3.5, 5.7, and 9.8. *D*: Insulin, quartile cut points: 9, 13, and 18.

AIR and increased split PI were significant predictors of diabetes (Table 3).

We examined the combined effects of low AIR and high intact or split PI concentration on risk of incident diabetes by including in the same logistic regression model both AIR and either intact or split PI (Table 4). The results demonstrated that AIR and intact (or split) PI were significant independent predictors of diabetes. We further examined these combined effects by stratifying study subjects at the median value of each of these variables. In logistic regression analyses of these groups, individuals with high AIR and low intact or split PI served as the reference group. Among subjects at or above the median for AIR, those in the high intact PI subgroup had a nonsignificant 1.4-fold increased risk of diabetes. Conversely, individuals with low AIR were at significantly elevated risk of diabetes regardless of their intact PI concentration. The low AIR/low intact PI group had an approximate threefold increased risk (OR 2.87, 95% CI 1.26–6.54), whereas the risk associated with low AIR and

TABLE 2
Multiple logistic regression analysis of measures of β-cell function and 5-year risk of type 2 diabetes in IRAS, adjusted for age, sex, ethnicity, and clinic

Independent variable	Stratification variable	Adjusted for age, sex, ethnicity, and clinic			Interaction term
		OR*	95% CI	χ^2	*P* value
AIR					
	Ethnicity				
	NHW	0.25	0.12–0.53	13.1†	0.45
	Black	0.42	0.20–0.74	8.9‡	
	Hispanic	0.39	0.24–0.64	13.6†	
	OGTT				
	NGT	0.55	0.34–0.89	6.0§	0.31
	IGT	0.40	0.25–0.64	14.4‖	
	Waist				
	<Median	0.28	0.14–0.55	13.6†	0.46
	≥Median	0.34	0.23–0.50	28.4‖	
	S_1				
	<Median	0.41	0.29–0.57	27.2‖	0.01
	≥Median	0.12	0.04–0.37	13.3†	
Intact PI					
	Ethnicity				
	NHW	3.82	2.51–5.84	38.7‖	0.002
	Black	1.38	1.02–1.86	4.3§	
	Hispanic	1.60	1.22–2.10	11.4‡	
	OGTT				
	NGT	2.21	1.63–3.02	25.6‖	0.014
	IGT	1.35	1.07–1.69	6.7‡	
	Waist				
	<Median	2.43	1.45–4.07	11.3†	0.35
	≥Median	1.62	1.32–2.00	20.5‖	
	S_1				
	<Median	1.48	1.22–1.78	15.3‖	0.49
	≥Median	2.68	1.53–4.70	12.0†	
Split PI					
	Ethnicity				
	NHW	3.06	2.12–4.42	35.6‖	0.07
	Black	1.38	1.05–1.81	5.5‡	
	Hispanic	1.94	1.42–2.65	17.2‖	
	OGTT				
	NGT	1.90	1.48–2.44	25.2‖	0.33
	IGT	1.55	1.21–1.98	12.1‡	
	Waist				
	<Median	1.91	1.23–2.97	8.2‡	0.81
	≥Median	1.76	1.44–2.15	30.2‖	
	S_1				
	<Median	1.61	1.34–1.94	25.1‖	0.96
	≥Median	2.10	1.11–3.97	5.2§	
Insulin					
	Ethnicity				
	NHW	2.80	1.79–4.38	20.5‖	0.28
	Black	1.13	0.83–1.53	0.6	
	Hispanic	1.75	1.23–2.45	10.6‡	
	OGTT				
	NGT	1.86	1.32–2.64	12.3‡	0.12
	IGT	1.26	0.99–1.59	3.6	
	Waist				
	<Median	1.39	0.90–2.16	2.2	0.79
	≥Median	1.49	1.14–1.93	8.8‡	
	S_1				
	<Median	1.66	1.25–2.21	12.3†	0.02
	≥Median	0.95	0.59–1.52	0.6	

Analyses were stratified by potential effect-modifying variables, including ethnicity, baseline IGT, baseline abdominal adiposity, and baseline insulin resistance. *ORs refer to risk associated with a 1 SD increase in the independent variable. †$P < 0.001$; ‡$P < 0.01$; §$P < 0.05$; ‖$P < 0.0001$. Definition of strata of potential effect modifiers: ethnicity (non-Hispanic white [NHW], black, Hispanic [His]), IGT (no/yes), abdominal adiposity (less than versus equal to or greater than the sex-specific median for waist circumference [males = 94 cm, females = 85 cm]), and insulin resistance (less than versus equal to or greater than the median for S_1 [1.64 min · μU^{-1} · ml^{-1}]).

TABLE 3
Multiple logistic regression analysis of baseline measures of β-cell function and risk of incident diabetes in the IRAS

Independent variable	OR	95% CI	χ^2	*P* value
AIR	0.32	0.22–0.48	32.3	<0.0001
Intact PI	0.93	0.66–1.30	0.2	0.66
Split PI	1.70	1.19–2.44	8.5	0.0036
Insulin	1.14	0.94–1.38	1.8	0.18

Results are presented with AIR, intact PI, split PI, and insulin included in the same model, with adjustment for age, sex, ethnicity, clinic, BMI, IGT, and S_1. ORs refer to risk of diabetes per SD increase in independent variable.

high intact PI was greater than fivefold (OR 5.65, 95% CI 2.67–11.97). These patterns of association were similar in an analysis using split PI in lieu of intact PI (low AIR/high split PI versus reference group: OR 6.16, 95% CI 2.72–13.96) (Fig. 2). However, in a separate analysis using continuous variables, neither the AIR–intact PI nor the AIR–split PI interaction terms reached statistical significance ($P = 0.27$ and $P = 0.17$, respectively).

DISCUSSION

In the present study, we demonstrated that both decreased AIR and increased PI concentration (either intact or split) were significantly associated with the 5-year incidence of diabetes after adjustment for confounding factors, including a detailed measure of insulin sensitivity. The findings were similar when analyses were conducted separately by baseline glucose tolerance status. In addition, we found that combinations of insulin secretion variables (AIR and either intact or split PI) independently and significantly predicted the development of diabetes in fully adjusted multivariate models. It is important to note the shortness of the follow-up period in this study relative to the decades required for the development of diabetes in middle age. Examination of subjects at a greater interval before the development of diabetes may have yielded a different set of predictors.

The results of the present article are consistent with a number of previous prospective studies that have shown that measures of insulin secretion, including AIR, were associated with risk of diabetes (2,6,7,9). In contrast, AIR was not associated with risk of progression to diabetes in a study from the Joslin clinic (41). The reasons for these inconsistencies are unclear, although in the Joslin study (41) the importance of insulin secretion may not have been detected because these investigators did not adjust for insulin sensitivity (Table 3) (6,7,9). A major contribution of the present study in terms of AIR is the demonstration that this variable is a significant prospective risk factor for diabetes, independent of confounding factors, within strata of ethnicity, glucose tolerance, abdominal adiposity, and insulin sensitivity. There was some suggestion of an interaction effect between AIR and insulin sensitivity level,

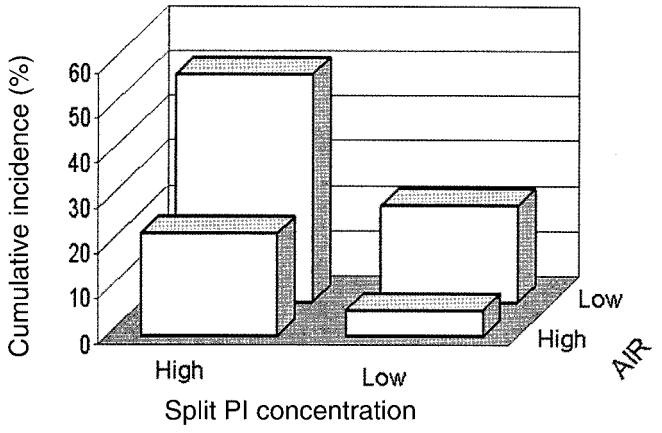

FIG. 2. Combined effects of low AIR and elevated split PI concentration on the 5-year risk of type 2 diabetes in IRAS. "High" and "low" defined by median values: AIR = 374.4 pmol · ml^{-1} · min^{-1}; split PI = 5.7 pmol/l. Figure shows 5-year cumulative incidence of diabetes in each of the four strata. In logistic regression analysis of these groups, adjusted for age, sex, ethnicity, clinic, BMI, IGT, and S_I; subjects with high AIR and low split PI serve as the reference category (OR 1.0). Subjects with high AIR and high split PI (OR 1.57, 95% CI 0.67–3.70); subjects with low AIR and low split PI (OR 3.20, 95% CI 1.35–7.62); and subjects with low AIR and high split PI (OR 6.16, 95% CI 2.72–13.96) are shown. *P* value for interaction using AIR and intact PI as continuous variables = 0.17.

with a stronger AIR–diabetes association among subjects with high S_I. A similar phenomenon was reported by Lillioja et al. (7) in a prospective study of Pima Indians.

Our observation that elevated baseline PI concentration, a measure of β-cell dysfunction, was a significant independent predictor of diabetes is consistent with previous studies that were conducted in populations of European white (18,19,23), Asian (17,22), Asian-American (20), and Mexican origin (21). The present results contribute to this body of literature in a number of ways. First, we have extended this finding to black and Hispanic Americans, large populations that experience moderate to high risk for type 2 diabetes (42). Second, we examined the prospective role of PI in the largest cohort of subjects with IGT studied to date and have confirmed that elevated PI is associated with increased risk of diabetes in this subgroup. Third, we used specific radioimmunoassays that measured concentrations of both intact and split PI. In two previous studies that used specific assays (19,23), there was the suggestion that split PI demonstrated a larger magnitude of association with risk of diabetes compared with intact PI. We also found this to be the case, although the differences in the ORs for these two variables were modest. Our finding that split PI but not intact PI was a significant predictor when the two were modeled together (Table 3) should be interpreted with caution, given the colinearity that exists between these two variables. More important, the association between split PI and risk of diabetes seemed to be modified less by ethnicity and baseline glucose tolerance status than the association between intact PI and risk of diabetes (Table 2). Finally, this is the first study to examine prospective associations of PI concentration after adjustment for a detailed measure of insulin sensitivity (S_I from the IGTT).

TABLE 4
Multiple logistic regression analysis examining the combined effect of AIR and intact PI (or split PI) on risk of incident diabetes in the IRAS

Independent variable	OR	95% CI	χ^2
(i) Model with intact PI*			
AIR	0.33	0.22–0.49	31.6†
Intact PI	1.45	1.16–1.83	10.2‡
(ii) Model with split PI*			
AIR	0.32	0.22–0.48	32.3†
Split PI	1.65	1.32–2.06	19.7†

Models are adjusted for age, sex, ethnicity, clinic, BMI, IGT, and S_I. ORs refer to risk of diabetes per SD increase in independent variable. *Areas under receiver operator characteristic curves: model (i) = 0.833; model (ii) = 0.837 (difference = NS). †$P < 0.0001$; ‡$P < 0.001$.

In the IRAS protocol, intact and split PI were measured only in the basal state and not at other time points during OGTT or FSIGTT. Kahn and Halban (43) demonstrated a close correlation between basal and 3-min arginine-stimulated PI concentrations in a sample of subjects with NGT and type 2 diabetes ($r = 0.88$, $P < 0.0001$). Stimulated concentrations measured during this time period are reliable because variations in peptide clearance rates will have "less impact on their relative levels in the circulation" (43). Thus, it seems that PI concentration in the basal state is a good surrogate measure for PI concentrations in conditions of acute β-cell stress.

The analytical convention in the PI literature is to "adjust" PI concentration for insulin secretion, usually by using the PI-to-insulin ratio. However, Kronmal (39) and Allison et al. (40) highlighted a number of potential statistical problems associated with the use of ratio variables, including undesirable effects on error distributions, the possible introduction of spurious correlations, and incomplete adjustment for the denominator variable. In the present analysis, both the intact PI–to–insulin and split PI–to–insulin ratios were significant predictors of diabetes, although the ORs and χ^2 statistics for these variables were more modest than those for intact and split PI adjusted by inclusion of insulin as a covariate in the multivariate model (data not shown). The greater reduction in the magnitude and significance of the associations with the use of ratio variables (intact PI–to–insulin and split PI–to–insulin) compared with the use of linear adjustment (with insulin) of intact and split PI suggested that the latter analytic strategy provided more precision.

We have shown for the first time that both low AIR and high PI independently predict the development of diabetes, an observation that has both mechanistic and public health implications. In terms of causative mechanism, the independence of these variables in the same multivariate models suggests the possibility that AIR and PI capture different aspects of impaired pancreatic β-cell function that are dually necessary for the development of diabetes. AIR during an FSIGTT measures the first-phase insulin response of the pancreatic β-cell mass to a glucose challenge. AIR is blunted or absent among individuals with diabetes (44), and derangements in AIR have been shown to predict the development of diabetes in nondiabetic subjects (2,6,7). It is conceivable, then, that AIR reflects the stored insulin reserve in the pancreatic β-cell mass. High concentrations of PI in diabetes, conversely, have been hypothesized to be due to changes in the clearance of PI (45), although recent evidence suggests that this is not the case (43). It has also been suggested that elevated PI is

the result of an abnormality in the conversion process of this prohormone (43). Mutations in the insulin gene that impair the conversion of PI to insulin have been reported to result in hyperproinsulinemia (46), although other evidence documenting conversion abnormalities is not available (43).

Alternatively, it is possible that either AIR or PI (or both) may reflect the overall health or volume of the pancreatic β-cell mass. It has been demonstrated that $AIR_{glucose}$ correlates strongly and significantly with directly measured β-cell mass and pancreatic insulin content ($r = 0.63$, $P < 0.02$; $r = 0.92$, $P < 0.001$; respectively) in baboons that had received varying doses of streptozocin (47). In addition, a reduced β-cell mass could conceivably result in elevated PI concentration, in that the rate of secretion by remaining cells is increased, thereby decreasing the intracellular stores and forcing the release of incompletely processed materials (43). Although it is unknown whether β-cell mass is reduced among prediabetic subjects, the lipotoxicity hypothesis of Unger (48) provides plausible indirect evidence. Under this hypothesis, elevated levels of free fatty acids (FFAs) cause in the short term β-cell hyperplasia and hyperinsulinemia, but with chronic exposure (and subsequent increase in FFA levels), they lead to functional and morphologic changes in β-cells. This notion has been supported with the documentation of substantial fat deposition in islets of obese Zucker rats and the demonstration of FFA-induced loss of glucose-stimulated insulin secretion (49). Increased FFAs also induce nitric oxide synthase, and Shimabukuro et al. (50,51) showed that elevated FFAs in rat β-cells cause increases in both nitric oxide levels and ceramide-mediated β-cell apoptosis (programmed cell death). It has been shown cross-sectionally in the IRAS population that both fasting and postchallenge FFA concentrations increase in a stepwise manner across worsening categories of glucose tolerance (52), and in other populations, it has been shown that increased FFA levels predict the development of diabetes (53,54).

Our findings also have implications for public health and clinical care. Although fasting PI concentration is more cost- and time-efficient to measure compared with AIR (which requires an FSIGTT), we found that AIR was a more powerful predictor of diabetes and, furthermore, that the combination of low AIR and elevated PI provided the best prediction of diabetes risk in this population. A number of ongoing clinical trials are examining the efficacy of lifestyle and various pharmacological agents (including metformin, acarbose, and the thiazoladinediones) in preventing diabetes in high-risk individuals (55–57). If the trials demonstrate the efficacy of these drugs or lifestyle approaches in preventing diabetes, then it may be beneficial to measure both AIR and PI in high-risk subjects, given the potential benefits of identifying for intensive intervention prediabetic individuals who would ultimately require many years of expensive pharmacological treatment.

In conclusion, we have documented the importance of decreased AIR and increased PI, both individually and in combination, in the development of type 2 diabetes. These findings highlight the central role of β-cell dysfunction in the early stages of the natural history of glucose intolerance and indicate the need for an increased understanding of the early determinants of β-cell abnormalities.

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