Relationship of β-Cell Function and Autoantibodies to Progression and Nonprogression of Subclinical Type 1 Diabetes

Follow-Up of the Seattle Family Study

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A total of 85 islet cell antibody (ICA)$^+$ or insulin autoantibody (IAA)$^+$ relatives of patients with type 1 diabetes have been followed as part of the Seattle Family Study for a mean of 2.8 years. Of the subjects followed, 10 developed diabetes during this time period. The presence of GAD antibodies was strongly associated with the development of diabetes. In contrast, the presence of IAAs did not influence the risk of diabetes among ICA$^+$ GAD$^+$ subjects. When either the initial absolute acute insulin response to glucose (AIR_g) or the AIR_g percentile, which accounts for the individual's insulin sensitivity, was below the 10th percentile of normal subjects, the risk of diabetes approached 50% at 5 years. However, impaired β-cell function did not influence the risk of diabetes among those who were GAD$^+$. There were 13 subjects with low AIR_g and 13 subjects with two or more antibodies who had not progressed to diabetes during the course of the study. Other measurements of β-cell function or demographic characteristics were not different in this group of nonprogressors compared with those with low AIR_g who did not progress to diabetes. We conclude that ICA$^+$ relatives with GAD antibodies or low AIR_g have a high risk for development of diabetes, but among ICA$^+$ GAD$^+$ relatives, the addition of IAA or a single determination of AIR_g does not enhance the prediction of diabetes. We suggest that prediction of diabetes risk depends on both the type and the number of antibodies present. In addition, there are a group of ICA$^+$ relatives with low AIR_g and/or multiple antibodies who have not progressed to diabetes over the course of the study.
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AIR_g, acute insulin response to glucose; AIR_t, acute insulin response to tolbutamide; IAA, insulin autoantibody; ICA, islet cell antibody; IDS, International Diabetes Society; IDW, International Diabetes Workshop; K_g, glucose disappearance constant; S_G, glucose effectiveness; S_I, insulin sensitivity index.

Studies of subjects before the onset of clinical type 1 diabetes have defined the risk of subsequent disease according to autoantibody measurements and β-cell function tests (1–7). Thus, relatives with multiple antibodies and/or low first-phase insulin secretion have up to a 50% risk of developing the disease over the next 3–5 years (8–11).

However, not all people who develop type 1 diabetes have autoantibody markers (i.e., the use of antibody markers alone results in a small percentage of false-negatives). Additionally, we and others found through prospective analysis of family members that not all antibody-positive individuals with abnormalities in β-cell function go on to develop diabetes (i.e., there are a significant number of false-positives) (6,8,12,13). This group has been termed "nonprogressors."

The goal of our study was to determine whether GAD antibody testing in addition to islet cell antibody (ICA) and insulin autoantibody (IAA) testing can better distinguish such nonprogressors from subjects who progress to clinical disease and to describe what β-cell function measurements are associated with progression to clinical disease.

RESEARCH DESIGN AND METHODS

All studies were approved by the University of Washington Human Subjects Review Committee, and informed written consent was obtained from all subjects before any studies. More than 3,000 first-degree relatives of people with type 1 diabetes were screened for the presence of ICAs and IAAs. A total of 85 subjects under 45 years of age had at least one of these antibodies on their screening test. These individuals were enrolled in the prospective family study for which they underwent antibody measurements and assessment of β-cell function (see below) about once a year.

ICA measurements. ICAs were measured by indirect immunofluorescence (6). Briefly, serum was preincubated with rat liver powder, and pieces of snap-frozen group O human pancreas were sectioned and incubated for 24 h with serum in doubling dilutions. Slides were washed in phosphate-buffered saline, and fluorescein isothiocyanate–conjugated goat anti-human immunoglobulin G was added. Positive and negative controls were included in each assay, and all samples were read in a masked fashion by two independent observers. ICAs were considered present if fluorescence was noted by both observers in two separate assays. We have participated in the International Diabetes Workshop (IDW) and the International Diabetes Society (IDS)-sponsored workshops and proficiency programs for ICAs with a sensitivity of about 63% and a specificity of 100%. The lower detection limit for our ICA assay is 1 Juvenile Diabetes Foundation unit.

IAA measurements. IAA levels were determined as previously described (14). Monoiodinated A14 human insulin tracer with an average specific activity of 300 μCi/μg was used with displacement by cold insulin. A subject was considered IAA$^+$ if the insulin specific binding was >3 SDs above the mean of normal control subjects (14). We have participated in the IDW and the IDS-sponsored

workshops and proficiency programs for IAAs with a sensitivity of about 100% and a specificity of 100%.

Intravenous glucose tolerance tests. After an overnight fast, an intravenous cannula was inserted into a peripheral forearm vein of each arm. To achieve arterialization of venous blood, the forearm of the arm used for blood sampling was placed inside a thermostatically controlled heating pad. Glucose and tolbutamide were infused through one cannula, while the other cannula was used to draw blood samples into EDTA for plasma glucose and insulin measurements. Patency of the intravenous sites was maintained by a slow infusion of 0.9% NaCl. After three basal blood samples were obtained (–10, –5, and –1 min), an intravenous bolus of 50% dextrose over 30 s was given followed by blood sampling at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, and 19 min. At 20 min, an intravenous bolus of 150 mg/m^2 of tolbutamide (Orinase; Upjohn, Kalamazoo, MI) was administered. Sampling continued at 22, 23, 24, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 min. Tolbutamide was used to enhance parameter identifiability by the minimal model of glucose kinetics (15).

Assays. Plasma insulin was assayed by a modification of the double-antibody method of Morgan and Lazarow (16). Plasma glucose values were measured by the glucose oxidase method with a glucose analyzer (Beckman, Palo Alto, CA).

Calculations. The insulin sensitivity index (S_I), which was calculated using the minimal model of glucose kinetics, provides an estimate of the ability of insulin to enhance glucose disposal (17). Glucose effectiveness (S_G) at basal insulin, which was calculated using the minimal model of glucose kinetics, provides an estimate of the ability of glucose to enhance its own disposal, independent of a change in insulin concentrations above basal (17).

The rate of glucose disappearance constant (K_g) was calculated as the natural logarithm of the glucose concentrations from 10 to 19 min. The acute insulin response to glucose (AIR_g; first-phase insulin release) was quantified as the mean incremental insulin response over the first 10 min after the glucose injection. A reference population of 93 normal subjects was used to determine the 10th percentile (18).

A curvilinear relationship between AIR_g and S_I has been defined and quantified from values based on 93 healthy subjects (18). Using these percentile plots, the values of AIR_g and S_I from each subject in the present study were used to determine the percentile for this relationship relative to the normal population (AIR_g percentile).

The acute insulin response to tolbutamide (AIR_t) was calculated as the incremental insulin response over the first 10 min after the tolbutamide injection. To account for the prevailing glucose level, the AIR_t was divided by the glucose value at the time of the tolbutamide injection to determine the AIR_t/glucose for comparisons between groups.

Statistics. Nonparametric Mann-Whitney U tests were used to compare continuous values of β-cell function tests at baseline between subjects who developed diabetes over the course of the study and those who did not. χ^2 analysis was done on dichotomous variables. Significance was accepted at the 5% level.

Life-table analysis (actuarial method) was used to estimate the 5-year cumulative diabetes-free survival for subjects grouped by baseline measurements.

RESULTS

Description of study group

Demographics. The 85 ICA$^+$ and/or IAA$^+$ subjects were followed up to 116 months with a mean of 33.9 months. There were 40 males and 45 females with a mean age of 24.1 years (range 5–45 years) at time of entry into the study (Table 1). Of our subjects, 46% were siblings, 33% were children, and 12% were parents of a diabetic proband; 11% had more than one family member with diabetes.

TABLE 1
Description of study subjects at baseline

	All subjects	Subsequent diabetes	No diabetes
Subjects (n)	85	10	75
Age (mean years; NS)	24. 2 (range 5–45)	18.5	21.9
M:F (n)	40:45	4:6	36:39
Length of follow-up (mean months; NS)	33.9	38.9	33.2

TABLE 2
Description of antibody markers

		GAD$^+$		GAD$^-$	
	Total	Subsequent diabetes	No diabetes	Subsequent diabetes	No diabetes
ICA$^+$	60	4	10	0	46
IAA$^+$	6	0	0	0	6
ICA$^+$ and IAA$^+$	19	6	9	0	4

Data are n.

Antibodies. Subjects entered the study if they were found to be ICA$^+$ or IAA$^+$ at their initial screening. Thus, at entry, 60 subjects were ICA$^+$, 6 were IAA$^+$, and 19 had both antibodies. GAD antibodies were subsequently measured at least once on each ICA$^+$ or IAA$^+$ subject. Of the subjects, 14 were GAD$^+$ and ICA$^+$, while an additional 15 had all three antibodies. There were no GAD$^+$ subjects who were IAA$^+$ and ICA$^-$ (Table 2).

β-Cell function. Of these antibody-positive relatives, 22% (19/85) had an AIR_g below the 10th percentile of a reference population of 93 normal subjects.

Antibodies and β-cell function. There was a high correlation between the presence of GAD antibodies and low AIR_g (Fisher's exact test, $P < 0.0001$) (Table 3). Of the GAD$^+$ subjects, 55% had low AIR_g compared with 5% of the GAD$^-$ subjects (Table 3).

Progressors

Antibodies. Ten subjects (12%) developed diabetes during follow-up (diagnosis of diabetes defined as clinical symptoms associated with hyperglycemia or a diabetic oral glucose tolerance test). All of these subjects were among the 29 individuals positive for both ICA and GAD antibodies (Table 2). In this group of ICA$^+$ and GAD$^+$ relatives, the addition of IAA$^+$ did not influence the risk for disease: 29% (4/14) of ICA$^+$ GAD$^+$ IAA$^-$ subjects progressed compared with 40% (6/15) of ICA$^+$ GAD$^+$ IAA$^+$ subjects (Fisher's exact test, $P = 0.70$). Prospectively, the estimated risk for development of diabetes among the GAD$^+$ relatives approached 50% at 5 years (Fig. 1).

β-Cell function. Subjects whose initial absolute AIR_g was below the 10th percentile of normal subjects were significantly more likely to develop diabetes than subjects whose AIR_g was above the 10th percentile. Of subjects with AIR_g below the 10th percentile, 32% (6/19) progressed compared with 6% (4/66) of subjects with AIR_g above this level (Fisher's exact test, $P < 0.007$). Similar results were seen when AIR_g percentile was used, i.e., 36% (5/14) of subjects below the 10th percentile developed diabetes compared with 5% (3/61) above this level (Fisher's exact test, $P < 0.005$).

TABLE 3
Initial β-cell function and the presence or absence of GAD in ICA$^+$ or IAA$^+$ relatives

	GAD$^-$	GAD$^+$	Total
AIR_g above 10th percentile	53	13	66
AIR_g below 10th percentile	3	16	19
Totals	56	29	85

Data are n. Fisher's exact test $P < 0.0001$.

TABLE 4
Comparison of baseline variables between those who subsequently did and did not develop diabetes

	Subsequent diabetes	No diabetes	*P* value
Fasting insulin (pmol/l)	68.4 ± 9.0	82.8 ± 4.6	0.27 (NS)
Fasting glucose (mmol/l)	5.3 ± 0.2	4.9 ± 0.1	0.09 (NS)
AIR_g (pmol/l)	125.2 ± 31.8	381.0 ± 29.4	<0.0005
AIR_g percentile	10.8 ± 5.1	43.3 ± 3.6	<0.005
AIR_t/glucose (pmol/l)/(mmol/l)	12.3 ± 2.2	36.0 ± 3.2	0.001
S_I ($\times 10^{-5}$ min^{-1}/[pmol/l])	4.47 ± 0.584	4.48 ± 0.443	0.56 (NS)
S_G (min^{-1})	0.015 ± 0.0016	0.022 ± 0.0009	0.01
K_g (%/min)	1.37 ± 0.311	1.94 ± 0.09	0.01

Data are means ± SD. *P* value from Mann-Whitney *U* test.

When expressed as continuous variables (Table 4), all measures of stimulated insulin secretion (AIR_g, AIR_g percentile, and AIR_t [adjusted for the glucose value at time of tolbutamide injection]) were significantly different between progressors and nonprogressors ($P < 0.005$ for all). S_G and K_g were also significantly lower for subjects who later developed diabetes compared with those who did not ($P = 0.01$ for both). In contrast, unstimulated fasting insulin, fasting glucose, and insulin sensitivity were not different in those who later developed diabetes.

Prospectively, when either absolute AIR_g (Fig. 2) or AIR_g percentile was below the 10th percentile, the estimated risk of development of diabetes was nearly identical and approached 50% at 5 years.

Antibodies and β-cell function. Among the ICA^+ GAD^+ subjects, progression to diabetes was seen in 6 of the 16 subjects with AIR_g below the 10th percentile and 4 of the 13 subjects with AIR_g above the 10th percentile (Fisher's exact test, $P > 0.99$). Thus, among ICA^+ GAD^+ subjects, low AIR_g did not discriminate progressors from nonprogressors (Table 5).

AIR_g over time. Four subjects who progressed to diabetes had AIR_g above the 10th percentile at initial study, and two of these subjects never fell below this level before their diabetes diagnosis. These data are illustrated in Fig. 3, which depicts AIR_g results over time in subjects who subsequently developed diabetes.

Nonprogressors. There were 75 subjects who did not progress to diabetes. Because low AIR_g or the presence of two or more antibodies was associated with progression to disease, we were particularly interested in subjects with one or both of these variables who did not develop diabetes.

Subjects with low initial AIR_g. Of the subjects, 13 had AIR_g below the 10th percentile of normal subjects at their baseline visit but had not progressed to diabetes after a mean of 30 months of follow-up. Of these subjects, 10 had ≥2 antibodies. In addition, in evaluating these subjects over time, there were no consistent patterns in change of AIR_t versus change of AIR_g over time that could distinguish this group's β-cell function from that of those who progressed to diabetes. Although some subjects seemed to improve their AIR_g over time, most continued to have low insulin secretion over the course of the study. These data are illustrated in Fig. 4*A*.

Subjects with initial AIR_g above the 10th percentile but with ≥2 antibodies. An additional 13 subjects with ≥2 antibodies had not progressed to diabetes after a mean of 26.8 months of follow-up. Nine of these subjects remained above

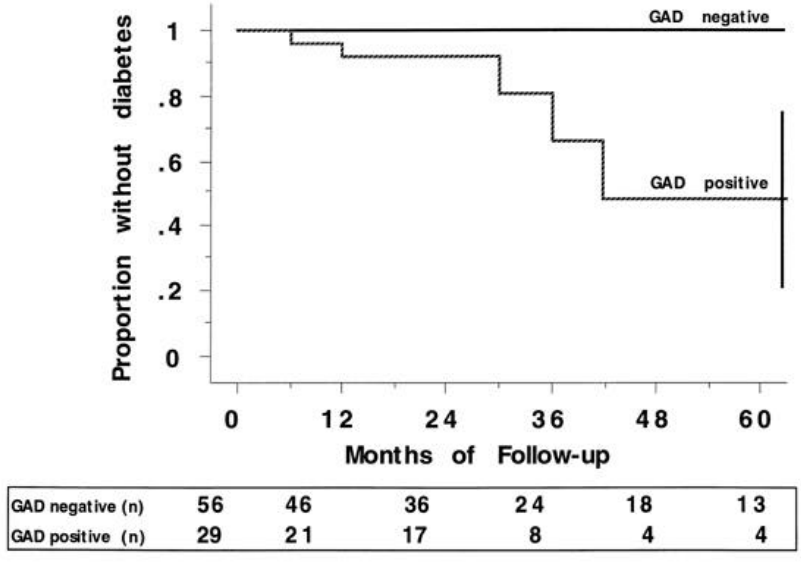

FIG. 1. The cumulative 5-year diabetes-free survival by GAD antibody status in ICA^+ or ICA^+ and IAA^+ relatives. Error bar represents the 95% CIs of cumulative diabetes-free survival.

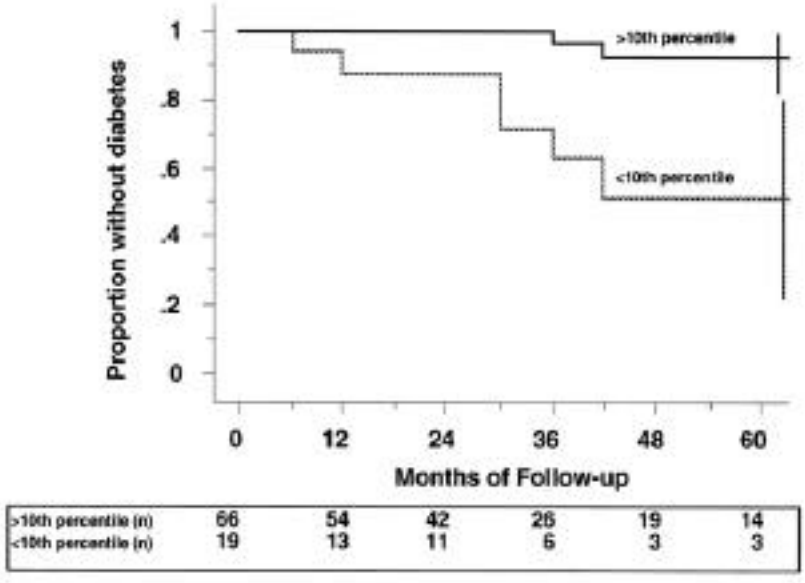

FIG. 2. The cumulative 5-year diabetes-free survival according to initial AIR_g ($P < 0.001$). Error bars represent the 95% CIs of cumulative diabetes-free survival.

the 10th percentile of AIR_g on all follow-up visits, while each of the remaining four had one measurement below the 10th percentile over time. There were four subjects who were ICA^+ and IAA^+ but GAD^-. These subjects, ages 11, 13, 14, and 36 at time of study entry, have not progressed to diabetes, and their initial and all but one follow-up AIR_g have remained above the 10th percentile. These data are illustrated in Fig. 4*B*.

DISCUSSION

Over the past decade, we have screened more than 3,000 relatives of patients with type 1 diabetes for the presence of ICAs or IAAs. Subjects who were positive for antibodies were enrolled in a prospective study to investigate the period of time before the onset of diabetes. Results from the first 5 years of this study have been previously reported (6). In this work, we report results on ICA^+ and/or IAA^+ subjects <45 years of age at the time of entry into the study. These 85 antibody-positive subjects have been followed for a mean of 2.8 years with at least annual measurements of autoantibodies and pancreatic β-cell function. During this time period, 10 subjects developed clinical type 1 diabetes.

The presence of GAD antibodies in the ICA^+ relatives was strongly associated with the development of diabetes, as all 10 of the subjects who developed diabetes were GAD^+. The presence of GAD antibodies in these ICA^+ relatives was also strongly associated with low β-cell function. Interestingly, although 6 of the 10 subjects who developed diabetes were IAA^+ as well as ICA^+ and GAD^+, the addition of IAA did not appear to influence the risk for disease. Thus, predictions based on the number of positive antibodies may have uneven performance depending upon which antibodies are positive. Similarly, impaired β-cell function did not influence the risk of diabetes among those who were GAD^+. Our data set suggests, therefore, that among ICA^+ GAD^+ relatives, the addition of IAA or a single determination of AIR_g does not enhance the prediction of diabetes. Although these data support the idea of using GAD in addition to ICA titer as an entry criteria for intervention trials, GAD antibodies were not the screening criteria for entry into the Seattle Family Study (i.e., GAD antibodies were measured only on relatives found to be initially ICA^+ or IAA^+). Therefore, this study did not determine the risk for development of diabetes among GAD^+ subjects who were

TABLE 5
Initial β-cell function and development of diabetes in subjects with ICA and GAD antibodies

	Subsequent diabetes	No diabetes	Total
AIR_g above 10th percentile	4	9	13
AIR_g below 10th percentile	6	10	16
Totals	10	19	29

Data are *n*. Fisher's exact test $P > 0.999$.

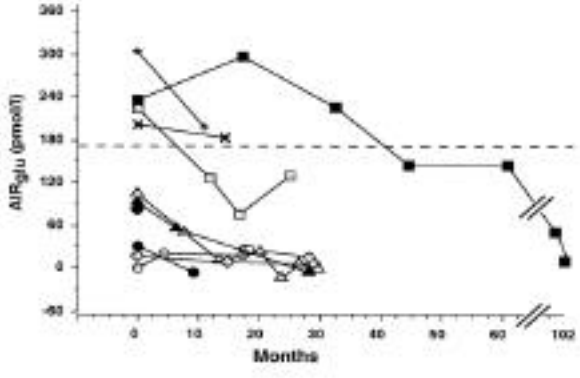

FIG. 3. AIR_g data over time in subjects who subsequently developed diabetes. — —, 10th percentile of a sample of 93 normal subjects (AIR_g 171 pmol/l) (18). Each symbol represents a different subject.

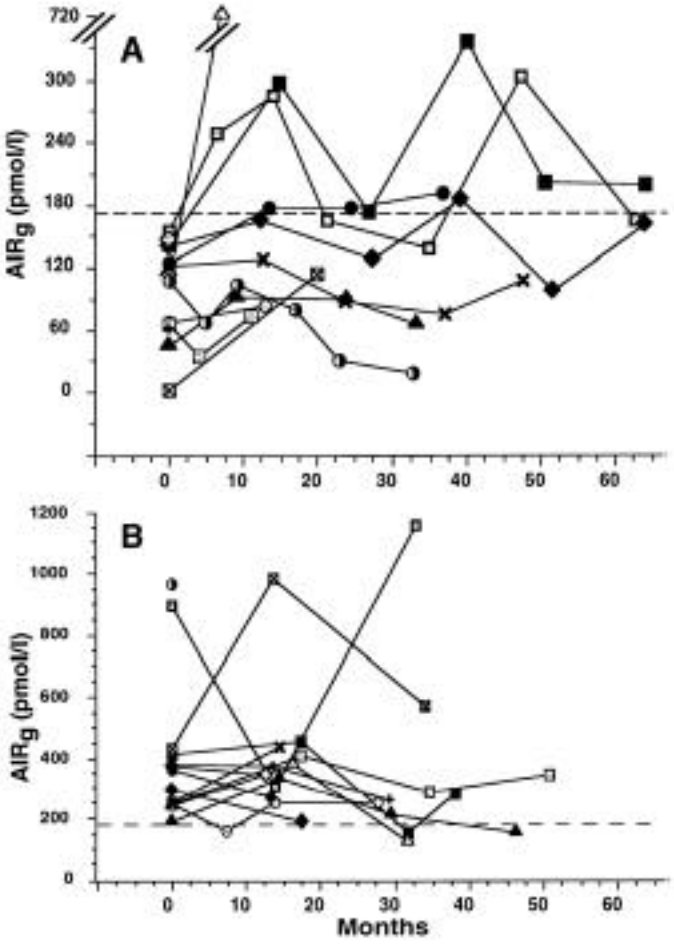

FIG. 4. AIR_g over time in subjects at high risk for diabetes who have not progressed to disease. *A*: Subjects with baseline AIR_g below the 10th percentile of normal subjects. *B*: Subjects with baseline AIR_g above the 10th percentile but with two or more antibodies. — —, 10th percentile of a sample of 93 normal subjects (AIR_g 171 pmol/l) (18). Each symbol represents a different subject. The four ICA^+ IAA^+ GAD^- subjects are indicted by the symbols □, +, ■, ⊠.

ICA^- or IAA^-. Other studies have suggested that the false-positive rate for GAD antibodies may be too high to use as a single screening test (19).

The question of how best to determine the physiological status of a subject with failing β-cells has been previously explored. Our current data indicate that some measure of stimulated insulin secretion is required to distinguish progressors from nonprogressors. Because an insulin-resistant subject will have greater insulin secretion than one who is insulin-sensitive, and conversely, lower insulin secretion would be seen in someone who is insulin-sensitive, we have previously suggested that measurement of stimulated insulin secretion alone does not fully reflect the physiology involved (6). In this study, we found that both the AIR_g and the AIR_g percentile were useful in predicting which subjects would go on to clinical disease, but that the use of the AIR_g percentile appears to offer no advantage over the much simpler measurement of AIR_g alone. This may be because the predictive value of insulin secretion for development of diabetes occurs only at very low levels of insulin secretion. Because insulin sensitivity did not differ between the progressors and nonprogressors, our data suggest that variations in insulin sensitivity do not alter the impact of markedly abnormal β-cell activity.

Our data also lend support to the concept that a severely impaired β-cell responds poorly to both glucose and tolbutamide, as subsequent development of diabetes was associated with low values for each parameter. In addition, although it has previously been reported that some β-cells that no longer normally respond to glucose can still maintain secretion to other stimuli (20–22), in this study, the AIR_t did not distinguish those subjects with low AIR_g who went on to develop diabetes from those who did not.

Our finding by survival analysis that low AIR_g is associated with an ~50% occurrence of diabetes within 5 years is absolutely consistent with reports from other centers and supports the use of these risk estimates in designing intervention trials (8–11). Although expressed somewhat differently, this increased risk appears to be less than that recently reported by Bingley for the ICARUS group (8). However, our data set includes all antibody-positive first-degree relatives who participated in the Seattle Family Study, whereas ICARUS initially focused on obtaining sera and information from subjects from around the world who progressed to diabetes. Consequently, ICARUS very likely had a selection bias with overrepresentation of subjects who subsequently developed diabetes.

Subjects who progressed to diabetes had on average a lower S_G than those who remained diabetes-free. Such a decrease in S_G has also been reported in subjects at risk for type 2 diabetes (23). Because this measurement of glucose disposal is independent of an increment in insulin above basal, this result suggests either that subjects who develop diabetes have primary defects in mechanisms responsible for glucose disposal or that the failure of insulin secretion subsequently alters glucose disposal by some indirect signal.

Finally, this study reconfirms our previous observations that a significant number of antibody-positive subjects with low AIR_g did not develop diabetes within the follow-up time available in this study (6). In fact, among GAD^+ ICA^+ subjects, low AIR_g was not associated with development of disease. We were unable to identify any differences in antibody status, age, or other β-cell function tests in these subjects to account for the apparent nonprogression of their disease. In addition, a significant number of subjects with two or more antibodies maintained good β-cell function over time and did not progress to diabetes. Conversely, some subjects whose AIR_g remained above the 10th percentile developed diabetes. Our data indicate that low AIR_g is associated with diabetes risk over the short term, i.e., a one-time measurement of low AIR_g is associated with developing diabetes, but if there are repeated tests indicating low AIR_g over time, the importance of this variable in predicting the development of disease decreases. As expected, however, and illustrated in Figs. 3 and 4, a persistent downward trend in AIR_g is generally seen in subjects before the development of diabetes, while the nonprogressors have a more variable pattern of β-cell function over time. The prospective data on these nonprogressors are consistent with the hypothesis that development of diabetes requires multiple "hits" (24). This idea suggests that although these subjects have clearly had some insult to their β-cells, they will not develop diabetes until another event occurs. Such an event may be a viral infection, as indicated by a

recent report demonstrating an association between an increase in antibody titers and development of diabetes (25,26). An alternative hypothesis to explain this data is that there is a continued progression of the disease process, but the progression is so slow that the subjects appear to be nonprogressors. This idea would suggest that these nonprogressing subjects may manifest diabetes in later years, perhaps as patients presenting with apparent type 2 diabetes but who are ICA^+ and/or GAD^+. We suggest that studies of T-cell activity and/or genetic analysis may provide further insights into this important group of subjects, potentially serving as models of how to arrest the autoimmune process.

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Author Queries (please see Q in margin and underlined text)

Q1: All authors from both Univ. of WA and DVA Puget Sound?

Q2: Fig. 4 legend, last sentence, please indicate which symbols should be included.