

Reduced Amylin Release Is a Characteristic of Impaired Glucose Tolerance and Type 2 Diabetes in Japanese Americans

Steven E. Kahn, C. Bruce Verchere, Sofianos Andrikopoulos, Pamela J. Asberry, Donna L. Leonetti, Patricia W. Wahl, Edward J. Boyko, Robert S. Schwartz, Laura Newell-Morris, and Wilfred Y. Fujimoto

Islet amyloid is a characteristic feature of type 2 diabetes. Its major component is the normal β -cell secretory product amylin, or islet amyloid polypeptide (IAPP). To determine whether increased or disproportionate release of amylin may explain the propensity for amyloid deposition in type 2 diabetes, we measured plasma amylin-like immunoreactivity (ALI) and immunoreactive insulin (IRI) release in response to an oral glucose load in 94 Japanese-American subjects with normal glucose tolerance (NGT; $n = 56$), impaired glucose tolerance (IGT; $n = 10$), and type 2 diabetes ($n = 28$) as defined by World Health Organization criteria. The incremental increase in ALI, IRI, and glucose (G) at 30 min after oral glucose ingestion was used to calculate Δ ALI/ Δ G and Δ IRI/ Δ G as measures of β -cell function. Overall glucose metabolism was assessed as the incremental glucose area (glucose AUC) during the 2 h of the oral glucose tolerance test. As expected, plasma glucose concentrations at both fasting (NGT, 5.0 ± 0.4 ; IGT, 5.5 ± 0.1 ; type 2 diabetes, 6.2 ± 0.3 mmol/l; $P < 0.0001$) and 2 h (NGT, 6.7 ± 0.1 ; IGT, 9.4 ± 0.3 ; type 2 diabetes, 13.2 ± 0.5 mmol/l; $P < 0.0001$) were elevated in individuals with IGT and type 2 diabetes. In response to glucose ingestion, plasma IRI and ALI increased in all subjects, but these increments were lower in individuals with reduced glucose tolerance, as reflected in the Δ IRI/ Δ G (NGT, 119 ± 10.3 ; IGT, 60.7 ± 7.1 ; type 2 diabetes, 49.7 ± 5.4 pmol/l; $P < 0.0001$) and Δ ALI/ Δ G (NGT, 2.6 ± 0.2 ; IGT, 1.8 ± 0.3 ; type 2 diabetes, 1.2 ± 0.1 pmol/l; $P < 0.0001$). Moreover, these reductions in the 30-min incremental ALI and IRI responses were proportionate such that the molar ratio of ALI to IRI was not different among the three groups (NGT, 2.6 ± 0.2 ; IGT, 2.9 ± 0.3 ; type 2 diabetes, $2.9 \pm 0.3\%$; NS). Further, the relationship between β -cell function, measured as either Δ IRI/ Δ G or Δ ALI/ Δ G, and glucose metabolism, assessed as glucose AUC, was nonlinear and inverse in

nature, with r^2 values of 0.38 ($P < 0.0001$) and 0.33 ($P < 0.0001$), respectively. We conclude that the reduced β -cell function of IGT and type 2 diabetes includes proportionate reductions in both IRI and ALI release. Thus, it is unlikely that the development of islet amyloid in type 2 diabetes is the result of increased release of ALI. *Diabetes* 47:640-645, 1998

Type 2 diabetes is characterized by β -cell dysfunction manifest in part as a reduction in insulin release (1). This alteration can be demonstrated before the onset of clinical hyperglycemia, namely in subjects with impaired glucose tolerance (IGT; 2,3). It has been hypothesized that deposition of islet amyloid results in a reduction in islet mass, thus contributing to impaired insulin release (4-7). Islet amyloid has been shown to be present in animal models of type 2 diabetes at a stage when these animals have IGT (8,9). Although the presence of these islet deposits in patients with diabetes has been recognized for over 90 years (10), only within the past 10 years have these deposits been recognized to contain a novel 37-amino-acid peptide termed amylin, or islet amyloid polypeptide (IAPP) (11,12).

After the identification and sequencing of amylin, we and others demonstrated that amylin is a normal β -cell secretory product that is co-released with insulin (13-15). It has been postulated that increased amylin release may contribute to the pathogenesis of type 2 diabetes by leading to the formation of islet amyloid and/or by inducing insulin resistance and diminished insulin secretion (16). Limited human studies suggest that amylin levels are increased with obesity (17) and that it is present in plasma of individuals with type 2 diabetes at concentrations that are lower, similar, or higher than control subjects (14,18). Limited immunostaining studies in cats performed at a time when they have islet amyloid and IGT suggest that amylin production may be increased during the pathogenesis of the type 2 diabetes syndrome in these animals (9). Thus, although it is certain that amylin is important in islet amyloidogenesis, it is unclear whether increased amylin secretion occurs in type 2 diabetes either when the disease is fully established or during its development.

Because type 2 diabetes and IGT are very common among Japanese Americans living in King County, Washington (19,20), this population provides an ideal cohort in which to examine the relationship between glucose tolerance and amylin secretion. Therefore, we performed a cross-sectional study in 94 members of this population group to answer the

From the Divisions of Metabolism, Endocrinology and Nutrition (S.E.K., C.B.V., S.A., P.J.A., W.Y.F.), General Internal Medicine (E.J.B.), Gerontology and Geriatric Medicine (R.S.S.), Department of Medicine, Department of Anthropology (D.L.L., L.N.-M.), and Department of Biostatistics (P.W.W.), University of Washington and VA Puget Sound Health Care System, Seattle, Washington.

Address correspondence and reprint requests to Steven E. Kahn, MB, ChB, VA Puget Sound Health Care System (151), 1660 S. Columbian Way, Seattle, WA 98108.

Received for publication 20 October 1997 and accepted in revised form 9 December 1997.

ALI, amylin-like immunoreactivity; AUC, area under the curve; Δ G, change in glucose; IGT, impaired glucose tolerance; IRI, immunoreactive insulin; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; WHO, World Health Organization.

TABLE 1
Plasma glucose, IRI, and ALI levels during the OGTT based on glucose tolerance status

	NGT	IGT	Type 2 diabetes	<i>P</i> value	Age-adjusted <i>P</i> value
<i>n</i>	56	10	28		
Glucose (mmol/l)					
0-min	5.0 ± 0.04	5.5 ± 0.1‡	6.2 ± 0.3*	<0.0001	<0.0001
30-min	8.9 ± 0.2	9.8 ± 0.5	10.5 ± 0.4*	<0.0001	<0.0001
60-min	9.1 ± 0.2	11.6 ± 0.7‡	13.5 ± 0.4*†	<0.0001	<0.0001
120-min	6.7 ± 0.1	9.4 ± 0.3‡	13.2 ± 0.5*†	<0.0001	<0.0001
IRI (pmol/l)					
0-min	58.9 ± 4.2	72.4 ± 7.6	102.1 ± 16.7*	0.004	0.005
30-min	496 ± 39	331 ± 43	308 ± 30*	0.003	0.004
60-min	567 ± 48	489 ± 96	450 ± 53	0.3	0.4
120-min	343 ± 26	487 ± 28‡	729 ± 101*	<0.0001	<0.0001
ALI (pmol/l)					
0-min	5.3 ± 0.5	5.9 ± 1.2	6.7 ± 1.3	0.4	0.3
30-min	15.2 ± 1.0	14.5 ± 2.0	11.6 ± 1.4*	0.1	0.1
60-min	21.5 ± 1.3	23.5 ± 3.7	17.0 ± 1.7*	0.08	0.08
120-min	20.0 ± 1.2	28.2 ± 3.0‡	25.0 ± 2.2*	0.01	0.03

**P* < 0.05 for type 2 diabetes vs. NGT; †*P* < 0.05 for type 2 diabetes vs. IGT; ‡*P* < 0.05 for IGT vs. NGT.

question of whether amylin release is normal, increased, or decreased in subjects with varying degrees of glucose intolerance. In so doing, we were also able to examine the relationship between amylin and insulin release and whether this is altered in individuals with or at high risk of developing type 2 diabetes.

RESEARCH DESIGN AND METHODS

Subjects. The study population comprised 94 (38 men/56 women) Japanese Americans living in King County, Washington, who were being screened for an intervention study to determine the effect of lifestyle changes on glucose metabolism in individuals with IGT. No subject was taking medications known to affect glucose metabolism. Each subject gave informed written consent to participate in the study, which was reviewed and approved by the Human Subjects Review Committee at the University of Washington.

Study methods. Body adiposity was determined from the BMI, calculated as weight (kg)/height² (m). All subjects were classified as having normal glucose tolerance (NGT), IGT, or type 2 diabetes based on the results of a single standard 75-g oral glucose tolerance test (OGTT) using World Health Organization (WHO) criteria (21). This test was performed in the morning after a 10-h overnight fast.

Assays and calculations. All blood samples were drawn into tubes containing EDTA and kept on ice before being separated. After separation, plasma was stored at -70°C before being assayed. Plasma glucose was measured by an automated glucose oxidase method. Plasma immunoreactive insulin (IRI) was measured by radioimmunoassay using an assay that has inter- and intra-assay coefficients of variation of 12 and 8%, respectively. The antibody used in the insulin assay cross-reacts fully with proinsulin and its conversion intermediates (22). Plasma amylin levels were quantified using a two-site enzyme-linked immunoassay system developed by Amylin Pharmaceuticals using antibodies F002 and F025 (23). This assay measures glycosylated and nonglycosylated forms of the peptide (23,24). It has inter- and intra-assay coefficients of variation of <15 and <10%, respectively, with a minimum detectable concentration of 1.6 pmol/l. Each sample was measured in duplicate for IRI and in triplicate for amylin-like immunoreactivity (ALI).

Calculations and statistical analysis. The incremental glucose (ΔG), IRI (ΔIRI), and ALI (ΔALI) responses were calculated as the difference between the values 30 min after glucose ingestion and those before glucose intake. The trapezoidal rule was used to calculate the incremental area under the curve (AUC) for glucose for the duration of the OGTT.

Statistical analysis was performed using Statview SE + Graphics (Abacus Concepts, Berkeley, CA). Data are presented as means \pm SE. Comparison between groups was performed by analysis of variance except when variables were non-normally distributed, in which case the Mann-Whitney *U* test was performed for two-group comparisons and the Kruskal-Wallis test when more than two groups were compared. Correlations were performed by linear regression, and nonlinear associations were modeled with the power function, where the dependent variable was a function of a constant multiplied by the independent variable raised to a fitted value ($y = a \cdot x^b$). A *P* value of <0.05

was considered significant.

RESULTS

Glucose tolerance status and demographics. Using WHO criteria applied to a single OGTT, 56 (26 men/30 women) of the 94 subjects were classified as having NGT, 10 (4 men/6 women) as having IGT, and 28 (8 men/20 women) as having type 2 diabetes. As detailed in Table 1, subjects with type 2 diabetes had very mild diabetes, with their fasting plasma glucose level at 6.2 ± 0.3 mmol/l and their 2-h glucose concentration at 13.2 ± 0.5 mmol/l. Although the groups differed slightly in age (NGT, 52.1 ± 1.8 ; IGT, 63.9 ± 3.7 ; type 2 diabetes, 58.3 ± 2.1 years; *P* = 0.01; *P* < 0.05 for NGT vs. both IGT and type 2), they were well matched for body adiposity assessed as BMI (NGT, 25.0 ± 0.6 ; IGT, 24.7 ± 1.1 ; type 2 diabetes, 26.5 ± 1.0 kg/m²; *P* = 0.4).

Glucose, IRI, and ALI levels during the OGTT. Glucose, IRI, and ALI levels measured at 0, 30, 60, and 120 min during the OGTT are listed in Table 1. In response to glucose, both plasma IRI and ALI increased. The peak IRI response occurred at 60 min in the NGT and IGT groups and at 120 min in the type 2 diabetes group. The peak ALI levels occurred at similar time points as IRI for the NGT and type 2 diabetes groups but occurred at 120 min in the IGT group. In addition to the expected differences in glucose concentrations, significant differences in IRI levels were present at 0, 30, and 120 min, whereas ALI levels were significantly different at 120 min and bordered on being significantly different at 30 and 60 min.

Incremental glucose, IRI, and ALI responses during the OGTT. When evaluating the responses during the OGTT, a number of factors were taken into consideration. First, glucose is a potent stimulus for β -cell peptide release. Second, the fasting glucose levels differed among groups. Third, glucose concentrations during the OGTT and thus the stimulus to the β -cell differed between groups. Therefore, we determined the magnitude of the glucose stimulus and assessed the release of IRI and ALI relative to it. To do this, we examined the incremental response from 0 to 30 min in IRI (ΔIRI) and ALI

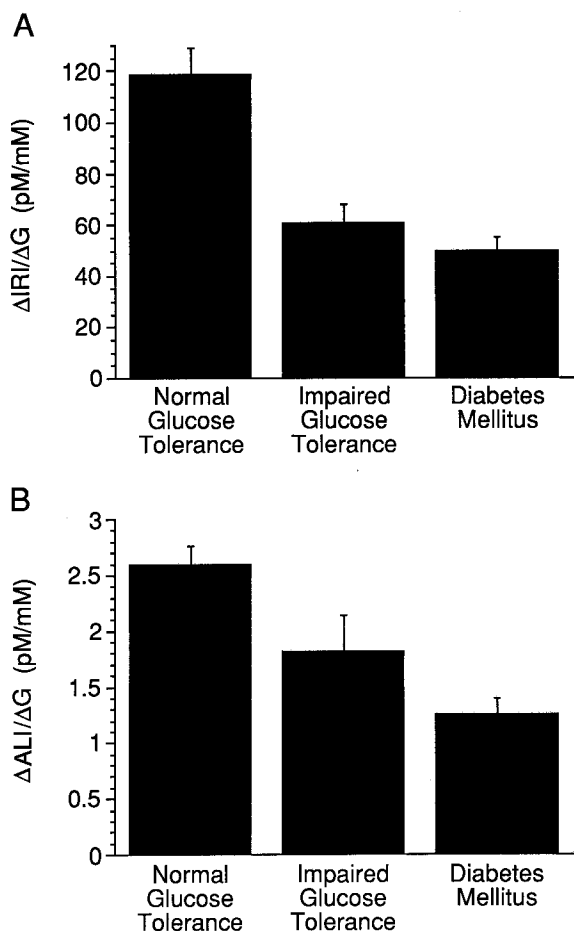


FIG. 1. Ratio of the incremental responses of IRI (A) and ALI (B) to the incremental glucose response over the first 30 min after oral glucose ingestion in 94 Japanese-American subjects with varying glucose tolerances. Of the 94 subjects, 56 had NGT, 10 had IGT, and 28 had type 2 diabetes. Significant decreases in both the $\Delta\text{IRI}/\Delta\text{G}$ ($P < 0.0001$) and $\Delta\text{ALI}/\Delta\text{G}$ ($P < 0.0001$) occurred with decreasing glucose tolerance.

(ΔALI) adjusted for the magnitude of the change in glucose (ΔG) for this time period. During the first 30 min after glucose ingestion, plasma glucose levels are increasing and very little of the glucose load is metabolized. Therefore, the $\Delta\text{IRI}/\Delta\text{G}$ and $\Delta\text{ALI}/\Delta\text{G}$ ratios provide a measure of β -cell responsiveness to oral glucose.

The incremental glucose response over the first 30 min did not differ between the three groups of subjects (NGT, 3.9 ± 0.2 ; IGT, 4.3 ± 0.4 ; type 2 diabetes, 4.3 ± 0.2 mmol/l; $P = 0.25$), although the absolute values tended to be higher in subjects with IGT and type 2 diabetes (Table 1). In contrast, the increment in IRI over this same time period differed significantly (NGT, 437 ± 37 ; IGT, 259 ± 40 ; type 2 diabetes, 205 ± 23 pmol/l; $P < 0.0001$; $P < 0.05$ for NGT vs. both IGT and type 2), as did the ALI increment (NGT, 9.9 ± 0.7 ; IGT, 7.6 ± 1.7 ; type 2 diabetes, 4.9 ± 0.5 pmol/l; $P < 0.0001$), primarily because of the lower responses in the subjects with type 2 diabetes ($P < 0.05$ vs. NGT). Thus, as illustrated in Fig. 1, β -cell responsiveness to glucose steadily declined as glucose tolerance deteriorated both for IRI as determined by $\Delta\text{IRI}/\Delta\text{G}$ (NGT, 119 ± 10.3 ; IGT, 60.7 ± 7.1 ;

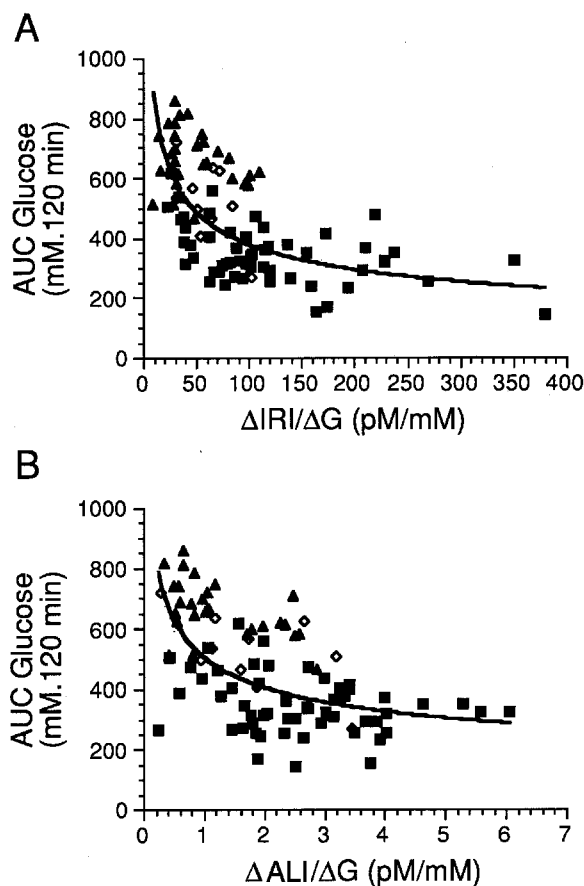


FIG. 2. Relationship between the ratio of the incremental IRI and glucose responses ($\Delta\text{IRI}/\Delta\text{G}$; A) and ALI and glucose responses ($\Delta\text{ALI}/\Delta\text{G}$; B) over the first 30 min after oral glucose ingestion to glucose tolerance, the latter determined as the incremental glucose area (AUC glucose) during the OGTT. Subjects with NGT (■), IGT (◇), or type 2 diabetes (▲) are indicated. The relationship between these variables are nonlinear, with r^2 values of 0.38 ($P < 0.0001$) and 0.33 ($P < 0.0001$), respectively.

type 2 diabetes, 49.7 ± 5.4 (pmol/l)/(mmol/l); $P < 0.0001$; $P < 0.05$ for NGT vs. both IGT and type 2) and for ALI as measured by $\Delta\text{ALI}/\Delta\text{G}$ (NGT, 2.6 ± 0.2 ; IGT, 1.8 ± 0.3 ; type 2 diabetes, 1.2 ± 0.1 (pmol/l)/(mmol/l); $P < 0.0001$; $P < 0.05$ for NGT vs. both IGT and type 2).

To determine the impact of β -cell function on glucose tolerance, we next examined the relationship between $\Delta\text{IRI}/\Delta\text{G}$ and glucose tolerance, the latter assessed as the incremental AUC for glucose during the duration of the entire OGTT. As would be expected, we observed a decline in the $\Delta\text{IRI}/\Delta\text{G}$ as glucose tolerance deteriorated, with the relationship between these two variables appearing to be nonlinear in nature (Fig. 2). Using a power fit, we were able to demonstrate a significant relationship between these two variables ($r^2 = 0.38$; $P < 0.0001$). It is also apparent from this figure that there is some degree of overlap between subjects classified based on WHO criteria; but in general, those with NGT have the highest responses and those with type 2 diabetes have the lowest responses. The relationship between $\Delta\text{ALI}/\Delta\text{G}$ and glucose AUC was similar in nature ($r^2 = 0.33$; $P < 0.0001$; Fig. 2) to that we observed for $\Delta\text{IRI}/\Delta\text{G}$ and glucose AUC, with there again being overlap

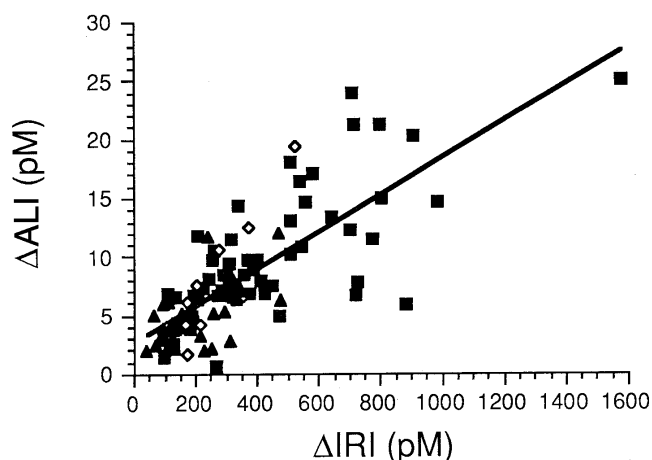


FIG. 3. Relationship between the incremental IRI (Δ IRI) and ALI (Δ ALI) responses over the first 30 min after oral glucose ingestion during the OGTT. Subjects with NGT (■), IGT (◇), or type 2 diabetes (▲) are indicated. These incremental responses are significantly related (slope = 0.016; $r = 0.76$; $P < 0.0001$).

between NGT and IGT individuals and IGT and type 2 diabetic individuals.

Relationship between ALI and IRI. In the fasting state, the molar ratio between ALI and IRI ($\text{ALI/IRI} \cdot 100$) declined in the type 2 diabetes group (NGT, 9.6 ± 0.9 ; IGT, 9.8 ± 0.2 ; type 2 diabetes, $6.3 \pm 0.5\%$; $P = 0.03$; $P < 0.05$ for both NGT and IGT vs. type 2). This reduced molar ratio in type 2 diabetes was due to the higher basal IRI levels in the subjects with the poorest glucose tolerance (Table 1). However, it is also important to recognize that since the clearance rates of amylin and insulin differ (17), this fasting ratio does not provide any information regarding the relative proportions of the peptides released in response to β -cell stimulation. Thus, to determine whether there may be some derangement in the relative proportions of ALI and IRI released, we also derived a ratio for the incremental responses of these two peptides between 0 and 30 min after oral glucose ingestion. As illustrated in Fig. 3, the release of these two peptides appears proportionate no matter what the degree of glucose tolerance is, with extensive overlap between the groups and with a correlation coefficient of 0.76 ($P < 0.0001$). The slope of the regression line for the relationship between Δ ALI and Δ IRI was 0.016, compatible with a molar ratio ($\Delta\text{ALI}/\Delta\text{IRI} \cdot 100$) of 1.6%. When the three groups were considered separately, the molar ratio in the first 30 min after glucose ingestion ($\Delta\text{ALI}/\Delta\text{IRI} \cdot 100$) were 2.6 ± 0.2 for NGT, 2.9 ± 0.3 for IGT, and $2.9 \pm 0.3\%$ for type 2 diabetes ($P = 0.5$).

Impact of body adiposity on ALI and IRI. Because increasing body adiposity is associated with a reduction in insulin sensitivity and insulin resistance is known to increase insulin release (25), we also examined the impact of BMI on IRI and ALI levels in all subjects, independent of their degree of glucose tolerance. As illustrated in Fig. 4, BMI correlated with both fasting IRI ($r = 0.63$; $P < 0.0001$) and fasting ALI ($r = 0.58$; $P < 0.0001$). No relationship existed between BMI and the fasting ALI/IRI ratio ($r = 0.004$; $P = 1.0$).

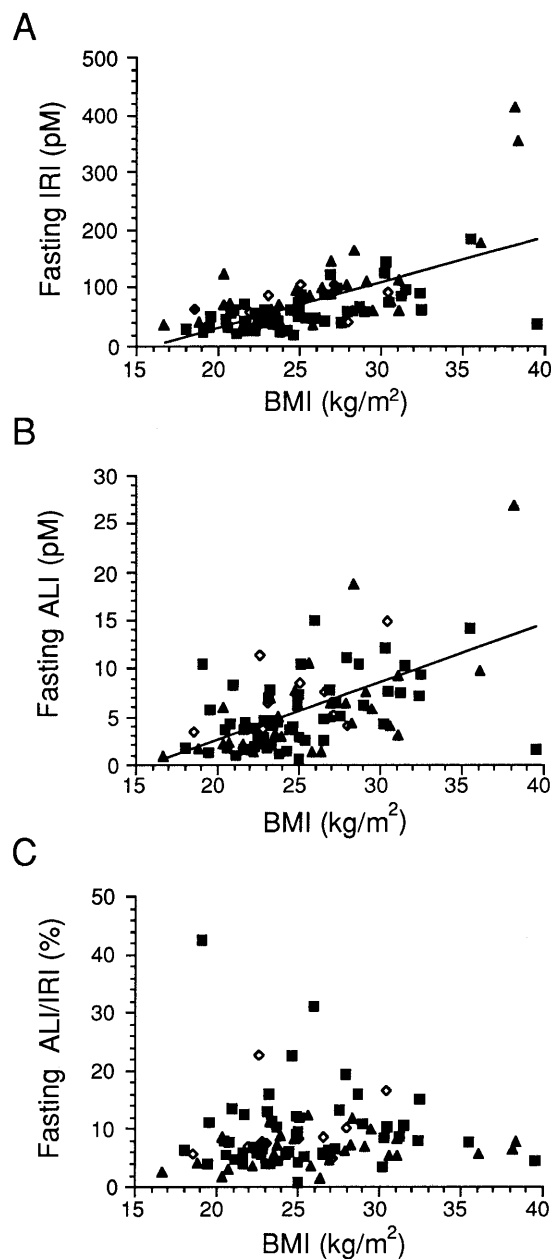


FIG. 4. Relationship between BMI and fasting plasma IRI (A), fasting plasma ALI (B) and fasting ALI/IRI (C) in 94 Japanese Americans with NGT (■), IGT (◇), or type 2 diabetes (▲). Both fasting IRI ($r = 0.63$; $P < 0.0001$) and fasting ALI ($r = 0.58$; $P < 0.0001$) increase with increasing BMI, whereas the fasting ALI/IRI does not ($r = 0.004$; $P = 1.0$).

DISCUSSION

In this study, we have demonstrated that oral glucose is capable of stimulating the release of amylin in addition to its well-recognized effect of increasing insulin release. This observation is not surprising, considering that amylin and insulin are co-localized in the same secretory granule within the β -cell (26) and are co-released in response to glucose and no-glucose secretagogues both in vitro (27) and in vivo (14,18). However, there are a number of unique aspects to the data we have collected from this cohort of Japanese Americans with varying glucose tolerance. First, by examining the relationship

between the ALI response and the magnitude of the stimulus provoking this response, we have been able to conclusively show that early amylin release, like insulin release, decreases with deteriorating glucose tolerance. Second, by examining the proportions of ALI and IRI circulating in plasma, we have found that differences in glucose tolerance and body adiposity are not associated with a discernible change in the molar proportions of these peptides.

It is now well recognized that type 2 diabetes is characterized by islet dysfunction that has impaired insulin secretion as one of its characteristic features. Much of the data demonstrating this has been obtained in studies using intravenous testing (1). Whereas the intravenous approach allows for the control of more variables capable of affecting β -cell function, it is not always feasible. Therefore, measures of β -cell function based on plasma measurements made following oral nutrient ingestion have been developed. These measures attempt to account for the variable rate of appearance in and clearance of the nutrient from the circulation, but they do not necessarily take into consideration the potential variability between subjects of the neurohormonal stimulus (28). One of the measures obtained from the OGTT that has been used extensively and demonstrated to correlate with the response to an intravenous glucose load is the ratio of the incremental insulin response to that in glucose over the first 30 min after oral glucose ingestion (3,29). Using this measure, we have again found that a reduction in the early phase of insulin secretion is characteristic of type 2 diabetes and is present before the development of clinical hyperglycemia. When using the same approach for ALI, we have conclusively demonstrated that a reduction in early amylin release is another feature of the β -cell dysfunction of type 2 diabetes. The finding of reduced amylin release is important, as it has been suggested that increased release of amylin may be responsible for certain pathophysiological components of type 2 diabetes (16). We believe that the present findings regarding amylin release apply to most populations with type 2 diabetes and IGT because Japanese Americans with these disorders of glucose metabolism also manifest both insulin resistance and β -cell dysfunction (3,30,31).

This study represents the first systematic examination of the relationship between amylin and insulin release in a large cohort of well-matched subjects with varying degrees of glucose tolerance. Previous studies have examined plasma amylin levels in small numbers of subjects with different degrees of glucose tolerance. In an early report, Butler et al. (14) demonstrated in healthy subjects and those with type 2 diabetes that amylin and insulin are released following ingestion of glucose or a mixed meal. In another early report, Mitsukawa et al. (32) found that both oral and intravenous glucose stimulated amylin release in healthy subjects, whereas somatostatin suppressed release of the peptide. In a study by Enoki et al. (33), obesity was associated with increased basal amylin levels and amylin responses to oral glucose and subjects with IGT had delayed but augmented amylin responses to oral glucose; in individuals with type 2 diabetes, the response was also delayed but lower. In two studies of patients with type 2 diabetes, subjects treated with insulin had lower basal amylin levels than did those taking sulfonylureas (18,34). Van Jaarsveld et al. (34) also showed that glucagon stimulated amylin release in patients with diabetes, but the levels attained were lower than those in healthy subjects.

Our data examining the molar proportion of ALI/IRI deter-

mined as the increment over 30 min following glucose ingestion confirm that the molar release of amylin is far less than that of insulin, being on the order of a couple of percent that of insulin. This ratio was observed without accounting for differences in peptide clearance. Amylin is known to be cleared more slowly than insulin, and its clearance rate is similar to C-peptide (17). Thus, it can be safely assumed that the proportion released when the secretory granule undergoes exocytosis is lower and is likely to approach 1% or less, as we have found in vitro and in islet extracts (13). This contrasts with the proportion following an overnight fast, which is higher and reflects the differences in clearance rates of the two peptides.

The observation of these low molar ratios is also of interest as it relates to the pathogenesis of the metabolic derangements of type 2 diabetes. It has been suggested that overproduction of amylin may be one of the underlying mechanisms responsible for islet amyloid formation (16). Studies have thus been performed in vitro examining amyloid fibril formation in cells transfected with human amylin (35). Although the data in the present study cannot directly answer whether simple overproduction or the release of granule content containing disproportionate amounts of amylin are responsible for amyloid development, they do suggest that neither of these mechanisms is operative. Rather, secretory granules released in response to stimulation most likely contain very similar proportions of amylin and insulin no matter what the degree of glucose tolerance, and the absolute quantities released are less with type 2 diabetes. It could be countered that such differences could have preceded the development of islet amyloid and that at the time we studied our subjects, the amyloid per se was inhibiting complete release of amylin into the circulation—thus our findings. However, based on a variety of other data, we believe that simple overproduction and release of amylin cannot explain islet amyloidogenesis in type 2 diabetes. First, obesity is associated with insulin resistance, which increases β -cell secretory demand (25) and results in increased amylin and insulin release as we have observed in the present study, yet obese individuals with NGT do not typically develop islet amyloid (36). Second, in work we and others have performed using transgenic mice, simple overproduction of amylin has not resulted in islet amyloid formation (37–41). Rather, islet amyloid only developed when mice were fed a high-fat diet, which we postulate to be producing an alteration in β -cell function (42,43). The exact changes in β -cell function induced by a high-fat diet are unclear, but they may be in part similar to the complex changes in β -cell function that occur in type 2 diabetes.

In conclusion, we have examined amylin release in Japanese Americans with varying degrees of glucose tolerance. These studies have demonstrated that like insulin release, the early phase of β -cell release of amylin is reduced in subjects with abnormal glucose tolerance, and the magnitude of this reduction is related to the degree of impairment in glucose tolerance. Further, the data strongly suggest that the relative proportions of amylin and insulin released do not change as glucose tolerance declines; thus, the data do not support the concept of disproportionate amylin secretion in type 2 diabetes.

ACKNOWLEDGMENTS

This work was supported in part by the Office of Research and Development, Medical Research Service, Department of Veterans Affairs, National Institutes of Health Grants DK-17047, DK-31170, DK-35816, DK-48152, DK-50703, and RR-00037, the

Medical Research Council of Canada, the American Diabetes Association, and the Juvenile Diabetes Foundation.

The comments of Daniel Porte, Jr., during the preparation of this manuscript are appreciated. We are grateful to Barbara Inglin, Maggie Abrahamson, Vicki Hoagland, Pam Yang, and the nurses of the General Clinical Research Center at the University of Washington for their care of the subjects and technical assistance. We are also grateful to the members of the King County Japanese-American community who participated in the study and to Amylin Pharmaceuticals for donating the assay kits.

REFERENCES

- Porte D Jr: β -cells in type II diabetes mellitus. *Diabetes* 40:166-180, 1991
- Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, Knowler WC, Bennett PH, Bogardus C: Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus: prospective studies of Pima Indians. *N Engl J Med* 329:1988-1992, 1993
- Chen K-W, Boyko EJ, Bergstrom RW, Leonetti DL, Newell-Morris L, Wahl PW, Fujimoto WY: Earlier appearance of impaired insulin secretion than of visceral adiposity in the pathogenesis of NIDDM: 5-year follow-up of initially non-diabetic Japanese-American men. *Diabetes Care* 18:747-753, 1995
- Maclean N, Ogilvie RF: Quantitative estimation of the pancreatic islet tissue in diabetic subjects. *Diabetes* 4:367-376, 1955
- Saito K, Yaginuma N, Takahashi T: Differential volumetry of A, B, and D cells in the pancreatic islets of diabetic and nondiabetic subjects. *Tohoku J Exp Med* 129:273-283, 1979
- Westermarck P, Wilander E: The influence of amyloid deposits on the islet volume in maturity onset diabetes mellitus. *Diabetologia* 15:417-421, 1978
- Clark A, Wells CA, Buley ID, Cruickshank JK, Vanhegan RI, Matthews DR, Cooper GJS, Holman RR, Turner RC: Islet amyloid, increased A-cells, reduced B-cells and exocrine fibrosis: quantitative changes in the pancreas in type 2 diabetes. *Diabetes Res* 9:151-159, 1988
- Howard CF Jr: Longitudinal studies on the development of diabetes in individual *Macaca nigra*. *Diabetologia* 29:301-306, 1986
- Johnson KH, O'Brien TD, Jordan K, Westermarck P: Impaired glucose tolerance is associated with increased islet amyloid polypeptide (IAPP) immunoreactivity in pancreatic beta cells. *Am J Pathol* 135:245-250, 1989
- Opie E: The relation of diabetes mellitus to lesions of the pancreas: hyaline degeneration of the islets of Langerhans. *J Exp Med* 5:527-540, 1901
- Westermarck P, Wernstedt C, Wilander E, Hayden DW, O'Brien TD, Johnson KH: Amyloid fibrils in human insulinoma and islets of Langerhans of the diabetic cat are derived from a neuropeptide-like protein also present in normal islets. *Proc Natl Acad Sci USA* 84:3881-3885, 1987
- Cooper GJS, Willis AC, Clark A, Turner RC, Sim RB, Reid KBM: Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients. *Proc Natl Acad Sci USA* 84:8628-8632, 1987
- Kahn SE, D'Alessio DA, Schwartz MW, Fujimoto WY, Ensinnck JW, Taborsky GJ Jr, Porte D Jr: Evidence of cosecretion of islet amyloid polypeptide and insulin by β -cells. *Diabetes* 39:634-638, 1990
- Butler PC, Chou J, Carter WB, Wang Y-N, Bu B-H, Chang D, Chang J-K, Rizza RA: Effects of meal ingestion on plasma amylin concentration in NIDDM and nondiabetic humans. *Diabetes* 39:752-756, 1990
- Cooper GJS: Amylin compared with calcitonin gene-related peptide: structure, biology, and relevance to metabolic disease. *Endocr Rev* 15:163-201, 1994
- Johnson KH, O'Brien TD, Westermarck P: Newly identified pancreatic protein islet amyloid polypeptide: what is its relationship to diabetes? *Diabetes* 40:310-314, 1991
- Kautzky-Willer A, Thomaseth K, Pacini G, Clodi M, Ludvik B, Strelci C, Waldhausl W, Prager R: Role of islet amyloid polypeptide secretion in insulin-resistant humans. *Diabetologia* 37:188-194, 1994
- Hartter E, Svoboda T, Ludvick B, Schuller M, Lell B, Kuenburg E, Brunnbauer M, Woloszk W, Prager R: Basal and stimulated plasma levels of pancreatic amylin indicate its co-secretion with insulin in humans. *Diabetologia* 34:52-54, 1991
- Fujimoto WY, Leonetti DL, Kinyoun JL, Newell-Morris L, Shuman WP, Stolov WC, Wahl PW: Prevalence of diabetes mellitus and impaired glucose tolerance among second-generation Japanese-American men. *Diabetes* 36:721-729, 1987
- Fujimoto WY, Leonetti DL, Bergstrom RW, Kinyoun JL, Stolov WC, Wahl PW: Glucose intolerance and diabetic complications among Japanese-American women. *Diabetes Res Clin Pract* 13:119-129, 1991
- WHO Expert Committee on Diabetes Mellitus: *Second Report*. Geneva, World Health Organization, 1980 (Tech Rep Ser, no. 646)
- Ward WK, Paquette TL, Frank BH, Porte D Jr: A sensitive radioimmunoassay for human proinsulin with sequential use of antisera to C-peptide and insulin. *Clin Chem* 32:728-733, 1986
- Percy AJ, Trainor DA, Rittenhouse J, Phelps J, Koda JE: Development of sensitive immunoassays to detect amylin and amylin-like peptides in unextracted plasma. *Clin Chem* 42:576-585, 1996
- Rittenhouse J, Chait BT, Bierle JR, Janes SM, Park DR, Phelps JL, Fineman MS, Qin J, Koda JE: Heterogeneity of naturally occurring human amylin due to glycosylation (Abstract). *Diabetes* 45 (Suppl. 2):235A, 1996
- Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, Neifing JL, Ward WK, Beard JC, Palmer JP, Porte D Jr: Quantification of the relationship between insulin sensitivity and β -cell function in human subjects: evidence for a hyperbolic function. *Diabetes* 42:1663-1672, 1993
- Lukinius A, Wilander E, Westermarck GT, Engstrom U, Westermarck P: Colocalization of islet amyloid polypeptide and insulin in the B cell secretory granules of the human pancreatic islets. *Diabetologia* 32:240-244, 1989
- Kahn SE, Fujimoto WY, D'Alessio DA, Ensinnck JW, Porte D Jr: Glucose stimulates and potentiates islet amyloid polypeptide secretion by the B-cell. *Horm Metab Res* 23:577-580, 1991
- Ward WK, Beard JC, Halter JB, Pfeifer MA, Porte D Jr: Pathophysiology of insulin secretion in non-insulin-dependent diabetes mellitus. *Diabetes Care* 7:491-502, 1984
- Phillips DI, Clark PM, Hales CN, Osmond C: Understanding oral glucose tolerance: comparison of glucose or insulin measurements during the oral glucose tolerance test with specific measurements of insulin resistance and insulin secretion. *Diabet Med* 11:286-292, 1994
- Bergstrom RW, Newell-Morris LL, Leonetti DL, Shuman WP, Wahl PW, Fujimoto WY: Association of elevated fasting C-peptide level and increased intra-abdominal fat distribution with development of NIDDM in Japanese-American men. *Diabetes* 39:104-111, 1990
- Fujimoto WY, Abbate SL, Kahn SE, Hokanson JE, Brunzell JD: The visceral adiposity syndrome in Japanese-American men. *Obes Res* 2:364-371, 1994
- Mitsukawa T, Takemura J, Asai J, Nakazato M, Kangawa K, Matsuo H, Matsukura S: Islet amyloid polypeptide response to glucose, insulin, and somatostatin analogue administration. *Diabetes* 39:639-642, 1990
- Enoki S, Mitsukawa T, Takemura J, Nakazato M, Aburaya J, Toshimori H, Matsukura S: Plasma islet amyloid polypeptide levels in obesity, impaired glucose tolerance and non-insulin-dependent diabetes mellitus. *Diabetes Res Clin Pract* 15:97-102, 1992
- van Jaarsveld BC, Hackeng WH, Lips CJ, Erkelens DW: Plasma concentrations of islet amyloid polypeptide after glucagon administration in type 2 diabetic patients and non-diabetic subjects. *Diabet Med* 10:327-330, 1993
- O'Brien TD, Butler PC, Kreutter DK, Kane LA, Eberhardt NL: Human islet amyloid polypeptide expression in COS-1 cells: a model of intracellular amyloidogenesis. *Am J Pathol* 147:609-616, 1995
- Clark A, Saad MF, Nezzet T, Uren C, Knowler WC, Bennett PH, Turner RC: Islet amyloid polypeptide in diabetic and non-diabetic Pima Indians. *Diabetologia* 33:285-289, 1990
- Fox N, Schrementi J, Nishi M, Ohagi S, Chan SJ, Heisserman JA, Westermarck GT, Leckstroem A, Westermarck P, Steiner DF: Human islet amyloid polypeptide transgenic mice as a model of non-insulin-dependent diabetes mellitus (NIDDM). *FEBS Lett* 323:40-44, 1993
- Hoppener JWM, Verbeek JS, de Koning EJP, Oosterwijk C, van Hulst KL, Visser-vernooy HJ, Hofhuis FMA, van Gaalen S, Berends MJH, Hackeng WHL, Jansz HS, Morris JF, Clark A, Capel PJA, Lips CJM: Chronic overproduction of islet amyloid polypeptide/amylin in transgenic mice: lysosomal localization of human islet amyloid polypeptide and lack of marked hyperglycaemia and hyperinsulinaemia. *Diabetologia* 36:1258-1265, 1993
- D'Alessio DA, Verchere CB, Kahn SE, Hoagland V, Baskin DG, Palmiter RD, Ensinnck JW: Pancreatic expression and secretion of human islet amyloid polypeptide in a transgenic mouse. *Diabetes* 43:1457-1461, 1994
- Westermarck G, Benig-Arora M, Fox N, Carroll R, Chan S-J, Westermarck P, Steiner DF: Amyloid formation in response to β cell stress in vitro, but not in vivo, in islets of transgenic mice expressing human islet amyloid polypeptide. *Mol Med* 1:542-553, 1995
- Verchere CB, D'Alessio DA, Kahn SE: Consequences of human islet amyloid polypeptide expression in transgenic mice. In *Lessons from Animal Diabetes VI: 75th Anniversary of the Insulin Discovery*. Shafrir E, Ed. Boston, Birkhäuser, 1996, p. 131-148
- Verchere CB, D'Alessio DA, Palmiter RD, Weir GC, Bonner-Weir S, Baskin DG, Kahn SE: Islet amyloid formation associated with hyperglycemia in transgenic mice with pancreatic beta cell expression of human islet amyloid polypeptide. *Proc Natl Acad Sci USA* 93:3492-3496, 1996
- Lee SK, Opara EC, Surwit RS, Feinglos MN, Akwari OE: Defective glucose-stimulated insulin release from perfused islets of C57BL/6J mice. *Pancreas* 11:206-211, 1995