# Hyperamylinemia Is Associated With Hyperinsulinemia in the Glucose-Tolerant, Insulin-Resistant Offspring of Two Mexican-American Non–Insulin-Dependent Diabetic Parents

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**Several investigations have presented evidence that amylin inhibits insulin secretion and induces insulin resistance both in vitro and in vivo. However, basal and postmeal amylin concentrations proved similar in non–insulin-dependent diabetes mellitus (NIDDM) patients and controls. Since hyperglycemia may alter both amylin and insulin secretion, we examined basal and glucose-stimulated amylin secretion in eight glucose-tolerant, insulin-resistant Mexican-American subjects with both parents affected with NIDDM (offspring) and correlated the findings with the insulin sensitivity data acquired by an insulin clamp. Eight offspring and eight Mexican-Americans without any family history of diabetes (controls) underwent measurement of fat free mass ($^3H_2O$ dilution method), 180-minutes, 75-g oral glucose tolerance test (OGTT), and 40-mU/m$^2$, 180-minute euglycemic insulin clamp associated with $^3$H-glucose infusion and indirect calorimetry. Fasting amylin was significantly increased in offspring versus controls (11.5 ± 1.4 *v* 7.0 ± 0.8 pmol/L, $P < .05$). After glucose ingestion, both total (3,073 ± 257 *v* 1,870 ± 202 pmol · L$^{-1}$ · min$^{-1}$, $P < .01$) and incremental (1,075 ± 170 *v* 518 ± 124 pmol · L$^{-1}$ · min$^{-1}$, $P < .05$) areas under the curve (AUCs) of amylin concentration were significantly greater in offspring. The amylin to insulin molar ratio was similar in offspring and controls at all time points. Basal and postglucose insulin and C-peptide concentrations were significantly increased in the offspring. No correlation was found between fasting amylin, postglucose amylin AUC or IAUC, and any measured parameter of glucose metabolism during a euglycemic-hyperinsulinemic clamp (total glucose disposal, 7.21 ± 0.73 *v* 11.03 ± 0.54, $P < .001$; nonoxidative glucose disposal, 3.17 ± 0.59 *v* 6.33 ± 0.56, $P < .002$; glucose oxidation, 4.05 ± 0.46 *v* 4.71 ± 0.21, $P$ = NS; hepatic glucose production, 0.29 ± 0.16 *v* 0.01 ± 0.11, $P$ = NS; all mg · min$^{-1}$ · kg$^{-1}$ fat-free mass, offspring *v* controls). In conclusion, these data do not support a causal role for amylin in the genesis of insulin resistance in NIDDM.**



BOTH INSULIN RESISTANCE[1,2] and insulin deficiency[3,4] have been implicated in the pathogenesis of non–insulin-dependent diabetes mellitus (NIDDM). Recently, islet amyloid polypeptide,[5] also called amylin,[6] a 37–amino acid peptide, has been isolated from amyloid deposits within pancreatic islets of NIDDM patients. Moreover, interstitial amyloid deposits have been shown to precede the onset of glucose intolerance in spontaneously diabetic monkeys.[7,8] In isolated rat islets,[9] in the perfused rat pancreas,[10] in the rat,[11] and in humans,[12] amylin has been shown to impair insulin secretion. Several studies also have shown that amylin is capable of inducing insulin resistance in vivo in both the liver and the skeletal muscle.[13-18] Recently, indirect evidence of the diabetogenic effect(s) of amylin has been provided by infusing rats with salmon calcitonin, which shares significant structural homology with amylin.[19] Therefore, it has been speculated that amylin could explain, at least in part, both disturbances, ie, impaired insulin secretion and insulin resistance, that characterize NIDDM. However, previous studies failed to demonstrate any acute effect of amylin on insulin secretion in vivo in rats and rabbits[20] and in mice and rats.[21] The observation by Butler et al[22] that basal and postmeal plasma amylin concentrations were similar in NIDDM and control subjects dampened the enthusiasm generated by the results of in vivo and in vitro studies in animals indicating that amylin played a role in the pathogenesis of NIDDM.[9-19,23] Since amylin and insulin are stored within and cosecreted by the β-cell, the possibility exists that the onset of hyperglycemia can alter amylin, as well as insulin, secretion. If this were to occur, studies of amylin secretion in overtly diabetic subjects with moderate to severe fasting hyperglycemia might not be indicative of the earliest stages of NIDDM when insulin secretion is not impaired. Studies conducted in the offspring of conjugal NIDDM individuals and in first-degree relatives of NIDDM subjects with normal glucose tolerance have demonstrated the presence of insulin resistance and a compensatory increase in insulin secretion.[24,25] Recently, we have demonstrated similar findings in the normal glucose-tolerant offspring of two NIDDM Mexican-American parents.[26] This group has a high incidence of developing NIDDM later in life. We therefore evaluated amylin secretion in the basal state and in response to an oral glucose load in our previously reported normal glucose-tolerant offspring of two diabetic parents.[26] This population, which is at high risk of developing NIDDM, allowed us to test the hypothesis that high basal or poststimulus amylin levels could be associated with insulin resistance in the early, preclinical stage of NIDDM.

## SUBJECTS AND METHODS

The study group consisted of 16 healthy Mexican-Americans with normal glucose tolerance as defined by the National Diabetes Data Group.[27] In eight individuals, both parents had documented NIDDM as defined by a fasting plasma glucose concentration greater than 7.8 mmol/L (140 mg/dL); this group is referred to as the offspring. Eight individuals without any family history of NIDDM served as controls.

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**Table 1. Clinical and Laboratory Characteristics in the Offspring and Controls**

| Characteristic | Offspring | Controls | P |
|---|---|---|---|
| No. of subjects | 8 | 8 | |
| Age (yr) | 40 ± 3 | 38 ± 4 | NS |
| Sex (M/F) | 2/6 | 1/7 | NS |
| BMI (kg/m²) | 26.3 ± 0.6 | 25.0 ± 1.1 | NS |
| FFM (kg) | 46.8 ± 3.8 | 46.4 ± 3.7 | NS |
| Fasting glucose (mmol/L) | 5.1 ± 0.2 | 4.9 ± 0.2 | NS |
| Fasting insulin (pmol/L) | 67 ± 4 | 46 ± 3 | <.02 |
| Fasting C-peptide (nmol/L) | 0.70 ± 0.09 | 0.44 ± 0.08 | <.05 |
| Fasting amylin (pmol/L) | 11.5 ± 1.3 | 7.0 ± 0.8 | <.05 |

NOTE. All values are the mean ± SEM.
Abbreviations: BMI, body mass index; FFM, fat-free mass.

All 16 of these subjects also participated in a study from which insulin sensitivity measurements have been previously reported.[26] Clinical and laboratory characteristics of the participants are shown in Table 1. Subjects consumed a weight-maintaining diet containing approximately 200 g carbohydrate per day for 7 days before study. Written informed consent was obtained from each subject. The study protocol was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio and the Radiation Safety Committee, General Clinical Research Committee, Research and Development Committee, and Radioactive Drug Research Committee of the Audie L. Murphy Memorial Veterans Affairs Hospital at San Antonio, TX.

On the day of study, subjects were admitted at 8:00 AM to the Diabetes Clinical Research Unit of Audie L. Murphy Memorial Veterans Affairs Hospital. All investigations were performed in the postabsorptive state after a 10- to 12-hour overnight fast. A 20-gauge Teflon catheter was inserted into an antecubital vein for blood sampling. After obtaining four baseline samples, each subject ingested 75 g glucose as an orange-flavored solution (Trutol 75; Oxford Labware, St Louis, MO) and blood samples were obtained at 30-minute intervals for 180 minutes. All samples were drawn into chilled polypropylene tubes containing EDTA (1 mg/mL) and aprotinin (500 U/mL), immediately centrifuged, and stored at −20°C until analyzed for plasma glucose, insulin, C-peptide, and amylin concentrations. At time zero, subjects also received an intravenous bolus of 80 μCi $^3H_2O$, and plasma samples for tritiated water radioactivity were obtained at 150, 165, and 180 minutes for determination of fat-free mass.[28] All subjects had previously received a 40-mU · min$^{-1}$ · (m$^2$)$^{-1}$ euglycemic insulin clamp[29] in combination with indirect calorimetry and [3-$^3$H]-D-glucose.

### *Analytical Determinations*

Plasma glucose concentration was determined in duplicate by the glucose oxidase method on a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Tritiated water radioactivity was determined by distillation as previously described.[26] Plasma insulin concentration was measured by a solid-phase $^{125}$I radioimmunoassay (RIA) (Coat-A-Count; Diagnostic Products, Los Angeles, CA). Plasma C-peptide was determined by a specific RIA (C-peptide $^{125}$I RIA Kit; Incstar, Stillwater, MN). Plasma amylin was determined as previously described.[13] Briefly, after elution through a Sep-Pac C-18 cartridge (Waters Chromatography Division, Millipore, Milford, MA), plasma amylin level was measured using a RIA kit (Peninsula, Belmont, CA) according to the manufacturer's specifications. Interassay and intraassay coefficients of variation were 11% ± 2% and 6% ± 1%, respectively.

### *Calculations*

Basal plasma concentrations of glucose, insulin, C-peptide, and amylin represent the mean of four values obtained during the baseline period (−30, −20, −10, and 0 minutes) of the oral glucose tolerance test (OGTT). The incremental areas under the curve (IAUCs) of plasma insulin, C-peptide, and amylin concentrations during the OGTT were calculated by the trapezoidal method.[30] All results are given as the mean ± SEM. The two-tailed unpaired Student's *t* test was used to test for statistically significant differences between groups. The two-tailed paired Student's *t* test was used to determine statistically significant changes from baseline within a group.

## RESULTS

The fasting plasma glucose concentration was similar in offspring and controls (5.1 ± 0.2 *v* 4.9 ± 0.2 mmol/L, $P$ = NS), and both groups showed normal oral glucose tolerance according to National Diabetes Data Group criteria[18] (Fig 1). Both fasting plasma insulin (67 ± 4 *v* 46 ± 3 pmol/L, $P < .02$; Fig 2) and C-peptide (0.70 ± 0.09 *v* 0.44 ± 0.08 nmol/L, $P < .05$; Fig 3) concentrations were significantly elevated in offspring versus controls. After glucose ingestion, there was a prompt increase in plasma insulin in both controls and offspring. The plasma insulin concentration was significantly greater in offspring versus controls (Fig 2). The plasma C-peptide response paralleled the plasma insulin response in offspring and controls (Fig 3). Both of the increments in plasma insulin (64,898 ± 4,862 *v* 34,687 ± 3,820 pmol · L$^{-1}$ · min, $P < .001$) and C-peptide (138 ± 46 *v* 60 ± 13 nmol · L$^{-1}$ · min, $P < .05$) areas above baseline were significantly greater in the offspring of NIDDM patients than in the controls. Fasting plasma amylin was significantly elevated in the offspring compared with the controls (11.5 ± 1.4 *v* 7.0 ± 0.8 pmol/L, $P < .05$). Within the first 30 minutes in the controls and 60 minutes in the offspring after glucose ingestion, the plasma amylin concentration remained unchanged. Thereafter, it increased progressively in both offspring and controls (Fig 4). After 90 to 120 minutes, plasma amylin declined in the controls, reaching basal levels within 3 hours. In contrast, in offspring plasma amylin continued to increase after 90 minutes, reaching a plateau between 150 and 180 minutes. Plasma amylin at time 120, 150, and 180 minutes was significantly increased in the offspring compared with the controls. Both the total AUC and IAUC of plasma

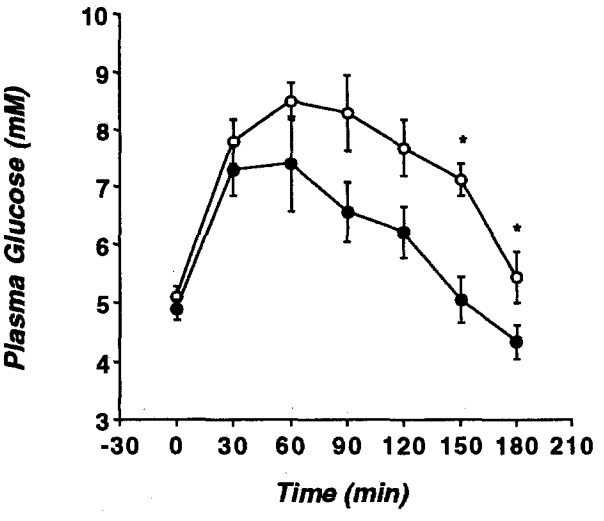


**Fig 1. Plasma glucose concentrations during the OGTT in controls (●) and offspring (○). Values are the mean ± SEM. *$P < .05$.**

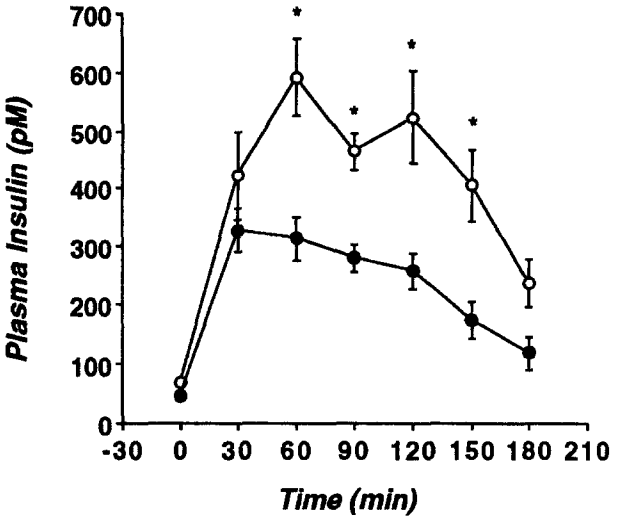


Fig 2. Plasma insulin concentrations during the OGTT in controls (●) and offspring (○). Values are the mean ± SEM. *$P < .05$.

amylin concentration were significantly greater in the offspring versus the controls (AUC, 3,073 ± 257 *v* 1,870 ± 202, and IAUC, 1,075 ± 170 *v* 518 ± 124 pmol · $L^{-1}$ · min, $P < .01$ and $<.05$, respectively). The amylin to insulin ratio (Fig 5) was similar in offspring and controls at all time points and showed the same time course after glucose ingestion. Neither the AUC or IAUC nor the individual time points of plasma insulin and plasma amylin concentrations during the OGTT showed a significant correlation. Insulin clamp data are reported in Table 2. No correlation was detected between fasting plasma amylin levels, postglucose plasma amylin AUC or IAUC, and any parameter of glucose metabolism during the insulin clamp: total-body glucose uptake ($r = .124$, $P$ = NS), nonoxidative glucose disposal ($r = .057$, $P$ = NS), glucose oxidation ($r = -.064$, $P$ = NS), and suppression of hepatic glucose production ($r = .397$, $P$ = NS).

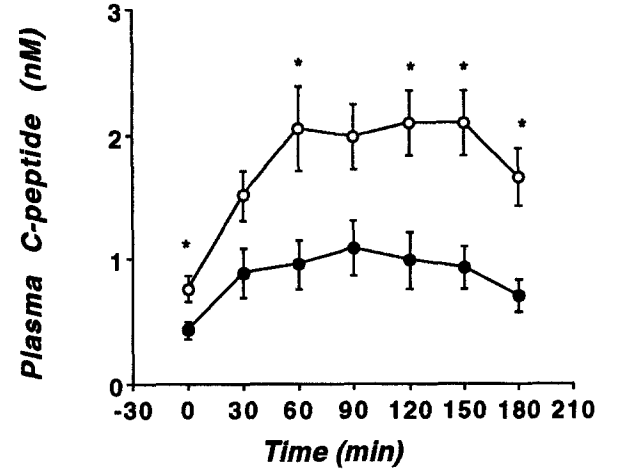


Fig 3. Plasma C-peptide concentrations during the OGTT in controls (●) and offspring (○). Values are the mean ± SEM. *$P < .05$.

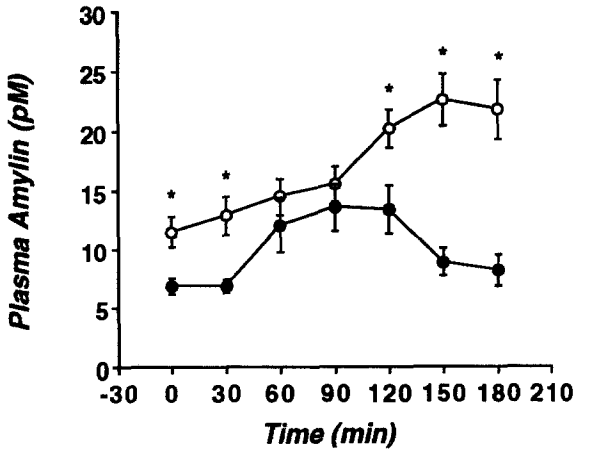


Fig 4. Plasma amylin concentrations during the OGTT in controls (●) and offspring (○). Values are the mean ± SEM. *$P < .05$.

## DISCUSSION

Amylin is a β-cell hormone that is cosynthesized, stored, and coreleased with insulin into the portal circulation in response to glucose and nonglucose stimuli.[22,31] Although the ratio between amylin and insulin has been reported to be fairly constant under both basal and stimulated conditions in nondiabetic animals[32] and man,[31] modifications in the amylin to insulin molar ratio have been reported in human NIDDM and in human obesity,[33] in obese-diabetic mice,[34] and in rats after treatment with streptozotocin[35] or dexamethasone.[36] These results indicate that in insulin-resistant states the synthesis and/or secretion of amylin are differently controlled,[36] and have led several investigators to consider amylin as a potential independent regulator of glucose metabolism. In normal-weight subjects with impaired glucose tolerance and in individuals with NIDDM, normal concentrations of amylin have been reported.[22,33] Since hyperglycemia can alter both amylin[35-37] and insulin[38,39] synthesis/secretion, we investigated basal and glucose-stimulated amylin levels in a selected population at high risk of developing NIDDM. We found significantly increased plasma amylin concentrations both in the fasting state and after glucose ingestion. These results are in contrast to those reported by

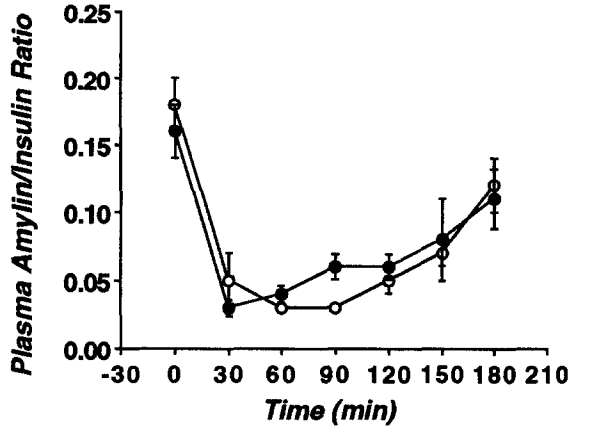


Fig 5. Plasma amylin/insulin ratio during the OGTT in controls (●) and offspring (○). Values are the mean ± SEM. *$P < .05$.

**Table 2. Insulin Clamp Data in the Offspring and Controls (mg · min$^{-1}$ · kg$^{-1}$ FFM)**

| Parameter | Offspring | Controls | P |
|---|---|---|---|
| TGD | 7.21 ± 0.73 | 11.04 ± 0.54 | <.001 |
| NOGD | 3.17 ± 0.59 | 6.33 ± 0.56 | <.002 |
| GOX | 4.05 ± 0.46 | 4.71 ± 0.21 | NS |
| RHGP | 0.29 ± 0.16 | 0.01 ± 0.11 | NS |

Abbreviations: TGD, total glucose disposal; NOGD, nonoxidative glucose disposal; GOX, glucose oxidation; RHGP, residual hepatic glucose production.

Eriksson et al[40] in first-degree relatives of patients with NIDDM. However, although in their study[40] basal plasma amylin levels were not statistically increased in first-degree relatives versus the control group (8 ± 1 *v* 9 ± 1 fmol/mL), the time course of plasma amylin concentration after glucose ingestion was different in the two groups. In the control group, plasma amylin began to decrease after 60 minutes, reaching a nadir at 120 minutes. In contrast, in the first-degree relatives plasma amylin continued to increase from 60 to 120 minutes and reached a plateau a 120 minutes. A similar time pattern of plasma amylin concentration has been reported by others after ingestion of glucose[33] or a mixed meal[22] in insulin-resistant or diabetic subjects. These results suggest that if their study[40] had been extended for 3 to 4 hours, a difference between the control and study population might have been detected. Indeed, if we had analyzed the amylin response (AUC or IAUC) during the 0- to 120-minute period, we would have failed to find any statistical difference between the offspring and controls. It also should be noted that whereas our offspring showed both basal and glucose-stimulated hyperinsulinemia, this finding was not a feature of the first-degree relatives studied by Eriksson et al.[40] Therefore, it could be argued that hyperamylinemia simply reflects the increased rate of insulin secretion. However, in our offspring, plasma amylin continued to increase from 120 to 180 minutes, whereas plasma insulin during the same time interval had returned to baseline values. This dichotomy could result from differences in the clearance of the two peptides or from a divergence in amylin and insulin synthesis/secretion during more prolonged stimulation. Indeed, Kautzky-Willer et al[41] have recently demonstrated that the fractional clearance rate of amylin was significantly slower compared with that of insulin both in control and in insulin-resistant (obese, hypertensive) subjects. They also showed that both conditions of insulin resistance are characterized by hyperinsulinemia and hyperamylinemia. Whether the amylin fractional clearance rate slows before or with the onset or worsening of insulin resistance remains to be evaluated.

As for the potential role of hyperamylinemia in the development of NIDDM, a number of effects of amylin on glucose metabolism have been reported with divergent results from both in vitro[42-44] and in vivo[45,46] studies. Studies in animals have shown that amylin inhibits insulin secretion[9,10] and reduces insulin-stimulated glucose metabolism[13,15,16] and insulin-mediated suppression of hepatic glucose production.[14] However, our in vivo studies[13] have shown that the antagonistic effects of amylin on both muscle and hepatic glucose metabolism require plasma concentrations in excess of 300 and 1,600 pmol/L, respectively. Such concentrations are more than 17- to 90-fold greater than those observed in the offspring in the present study. Moreover, Wilding et al[47] have shown that an approximately 100-fold elevation of plasma amylin concentration failed to acutely inhibit glucose metabolism during a euglycemic-hyperinsulinemic (~190 pmol/L) clamp. Consistent with this, we failed to find any correlation between fasting plasma amylin levels, plasma amylin AUC or IAUC, and any parameter of glucose metabolism during the insulin clamp. These observations do not support a causal role for amylin in the genesis of insulin resistance in NIDDM. Similarly, the plasma concentration of amylin that has been shown to inhibit insulin secretion is in the pharmacological range of 0.1 to 1 μmol/L.[9,10] This value is substantially higher than observed in the offspring in the present study. This finding contradicts the results of other studies,[13-19] although differences in experimental design (ie, in vivo *v* in vitro) and species differences in amylin structure and action (ie, human *v* rat) might account for the widely discrepant conclusions. Further investigations will be required to ascertain a possible pathophysiologic link between the observed hyperamylinemia and the pathogenesis of NIDDM in Mexican-Americans.

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