

Serum Amylin Indicates Hypertriglyceridemia in Pre-diabetics

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Abstract

Background: Hypertriglyceridemia is associated with increased insulin secretion and the incidence of Type 2 Diabetes. Studies in rodents and obese children have proposed a relationship between hypertriglyceridemia and elevated plasma amylin levels. However, there are limited data regarding serum amylin and triglyceride levels in adult human subjects with impaired glucose tolerance. In this study, we evaluated the association between serum triglycerides with circulating amylin levels in adult subjects with and without impaired glucose tolerance.

Methods: Glucose, insulin, Hemoglobin A1c, triglycerides, total amylin and unreduced amylin levels were determined in 80 non-pregnant females and 60 males enrolled in a cross-sectional study. Subjects were allocated according to their glucose tolerance status into normal glucose tolerance (n=81) and impaired glucose tolerance (n=59). Subjects with Type 2 Diabetes, endocrine diseases, chronic inflammation or inflammatory diseases, as well as alcoholism were excluded. Correlation and sensitivity between triglycerides and amylin was investigated using Pearson's correlation coefficient (r) and Receiver-Operating Characteristics (ROC) curve, respectively.

Results: Serum triglycerides strongly correlated with only total amylin (r=0.470, p<0.05) in the impaired glucose tolerance group and not the normal glucose tolerance group. No correlation between triglycerides and unreduced amylin was observed in neither group. The area under the ROC curve (AUC) was only significant for hypertriglyceridemia and total amylin in the impaired glucose tolerance group (AUC=0.731, 95% CI: 0.600-0.838, p<0.05).

Conclusion: Our results suggest that serum triglycerides and total amylin levels are significantly associated in impaired glucose tolerance subjects.

Keywords: Total amylin; Unreduced amylin; Hypertriglyceridemia; Pre-diabetes; IAPP

Introduction

Islet amyloid polypeptide (IAPP or amylin) is a 37 amino acid residue hormone expressed mainly by pancreatic islet β -cells. It is well-known for its ability to aggregate into amyloid fibrils in islets of Langerhans leading to loss of β -cells function [1-3], a step that is crucial in the progression from pre-diabetes to Type 2 Diabetes. Amylin, which is co-secreted with insulin, regulates post-prandial glucose levels in part by inhibiting gastric emptying and suppressing glucagon release while not affecting glucose uptake [4]. Amylin has been shown to mirror insulin secretion in healthy subjects [5], obese subjects, pre-diabetics (subjects with impaired glucose tolerance) [6,7], and patients with Type 2 Diabetes [5]. These findings suggest an active role for plasma amylin levels in modulating glucose metabolism [8]. However, the mechanisms and the precise role of amylin in the pathophysiology of Type 2 Diabetes remains unclear.

There have been multiple forms of amylin detected in plasma: unreduced amylin (presence of a disulfide bond between amino acid 2 and 7), reduced amylin, and amylin acid (deamidated amylin) [9]. Experimental studies have shown that infusion of amylin into rats lead to elevated triglyceride content in liver and muscle tissue [10] and that infusion of amylin-(8-37), a specific amylin antagonist, reduced muscle triglycerides. Mice, on a long-term high-fat diet, showed augmented amylin levels [1]. Indeed, the free fatty acids palmitate and oleate have been shown to increase amylin expression [11]. Amylin, in turn, blocked glucose disposal by liver cells [12] and exerted a lipolytic-like action leading to increased plasma non-esterified fatty acids

and glycerol levels [10]. These results strongly suggest an important relationship among triglycerides, free fatty acids, and amylin release.

Hypertriglyceridemia has been associated with increased insulin secretion in non-obese subjects, pre-diabetics [13,14], and patients with Type 2 Diabetes [15,16]. These results support the contention that hypertriglyceridemia may be related to amylin secretion. However, reports about the relationship between hypertriglyceridemia and plasma amylin levels are scarce [6,10,17]. Taken together, these observations prompted us to evaluate the association between serum triglycerides with circulating amylin levels among subjects with and without impaired glucose tolerance.

Material and Methods

Subjects and settings

Following approval from the Scientific Research and Ethics

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Committees of the Mexican Social Security Institute (IMSS) and the written informed consent of participants in accordance with the Declaration of Helsinki, a cross-sectional study was carried out. The study included males (n=60) and non-pregnant females (n=80), aged 18 to 77 years-old. The subjects were from urban and rural communities from Puebla, Mexico and met the following criteria: healthy and without Diabetes. Subjects were excluded from the study if they had other endocrine diseases, chronic inflammation or inflammatory diseases, as well as alcoholism.

Clinical characterization

Subjects were clinically evaluated according to a standardized protocol including personal and family history. In the standing position, weight and height were measured with the subjects in light clothing and without shoes using a fixed scale with stadimeter (Tanita TBF-215, Tokyo, Japan). Body Mass Index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. BMIs <18.4 Kg/m² and >40 Kg/m² were excluded. Total body fat was measured by bioelectric impedance using a body composition analyzer (Tanita TBF-215, Tokyo, Japan). Blood pressure was measured according to the recommended technique in the Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure [18]. The data is the average of two physician-obtained measurements.

Biochemical assays

Venous blood samples were collected into EDTA containing tubes after an overnight fasting of 10 to 12 hours. Blood samples were used for the follow endpoints: fasting glucose and insulin, oral glucose tolerance test (Glucose 2-h post load), Hemoglobin A1c, amylin (total and unreduced-form), and triglycerides. A new blood samples were obtained 2 h after oral glucose administration (75 gr) to determine the Glucose 2-h post load. Fasting glucose and Glucose 2-h postload were determined, in duplicate, using the enzymatic method/spectrophotometric glucose oxidation (Beckman Instruments, Brea, CA). Fasting insulin levels were determined by chemiluminescent immunoassay (Abbott). The Hemoglobin A1c levels were determined by the turbidimetric inhibition immunoassay. Plasma amylin levels were measured by ELISA following manufacturer's instructions (Millipore, Billerica, MA [EZHAT-51K for total amylin and EZHA-52K for unreduced form]). Total amylin corresponds to the unreduced and reduced forms, whereas the unreduced amylin corresponds to only the unreduced form. The sensitivity of the assay was 1 pM for amylin in our hands. The coefficient of variation of amylin was 1.8%. High and low control samples were run simultaneously with values accepted only when control values were within this range. Triglycerides were enzymatically measured using spectrophotometric methods; the intra- and inter-assay coefficients of variation were 1.7% and 3.1%. Normal values for triglycerides (<150 mg/dL) were defined according to NOM-037-SSA2-2002 [19].

Definitions

Using the American Diabetes Association recommendation [20], subjects were classified as either impaired glucose tolerance (fasting glucose: 100-126 mg/dL, Glucose 2-h postload: 140-200 mg/dL, or Hemoglobin A1c: 5.7-6.4%) or normal glucose tolerance (fasting glucose: <100 mg/dL, Glucose 2-h postload: <140 mg/dL, or Hemoglobin A1c: <5.7%).

Statistical analysis

Statistical analyses were performed using Statistical Package for

the Social Sciences program for Windows, version 19 (SPSS, Chicago, IL) or Medcalc Statistical Software version 13.3.3 (Medcalc, Ostend, Belgium). Differences between groups were evaluated using Student T-test. The results were expressed as mean \pm standard error (SPSS). Correlation analysis was done by calculating the Pearson's correlation coefficient (r). Receiver Operating Characteristic (ROC) curve was used to determine the sensitivity and specificity of amylin to determine hypertriglyceridemia. The area under the ROC curve (AUC) was calculated using the method described by Hanley and McNeil [21] (Medcalc). p-values <0.05 were considered statistically significant.

Results

A total of 80 non-pregnant females (57.1%) and 60 males (42.9%) were enrolled; of these, 81 (57.9%) and 59 (42.1%) were allocated into the normal glucose tolerance group and the impaired glucose tolerance group, respectively. The clinical and metabolic characteristics are summarized in Table 1. There was no difference in triglycerides, total amylin, or unreduced amylin concentrations between the normal glucose tolerance group and the impaired glucose tolerance group. However, when the relationship between triglycerides and total amylin levels was determined, only the impaired glucose tolerance group demonstrated a strong correlation (r=0.470, p<0.05, Figure 1B). No correlation between total amylin and triglycerides was seen with the normal glucose tolerance group or between unreduced amylin and triglycerides for both groups (Figure 1A, C, and D). For total amylin, the impaired glucose tolerance group did specifically determine hypertriglyceridemia (AUC=0.731, 95% CI: 0.600-0.838, p<0.001, Figure 2B), which was not seen with the normal glucose tolerance group (AUC=0.587, 95% CI: 0.472-0.635, p=0.185, Figure 2A). With both groups, unreduced amylin could not specifically determine hypertriglyceridemia (normal glucose tolerance: AUC=0.605, 95% CI: 0.469-0.730, p=0.168 and impaired glucose tolerance: AUC=0.523, 95% CI: 0.409-0.635, p=0.729).

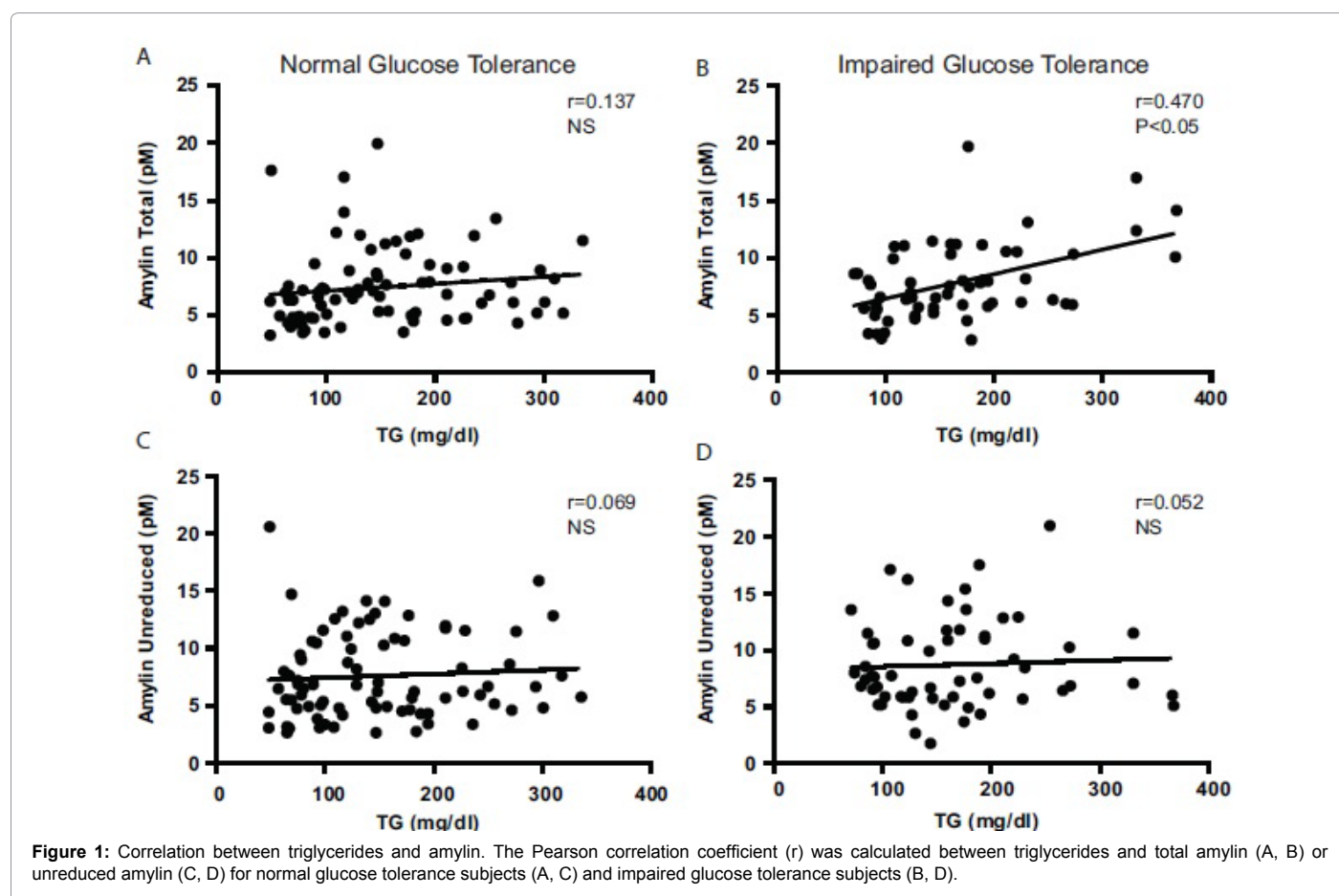
Discussion

Our results show a significant association between serum triglycerides and total amylin levels among pre-diabetics; findings that support the contention that hyperamylinemia may contribute to the pathogenesis of Type 2 Diabetes. Other reports observed increased circulating amylin levels in pre-diabetics [1,22,23]. However, in accordance with Butler et al., our results failed to observe any appreciable difference in amylin levels between pre-diabetics and control subjects [24]. Consequently, given that insulin and amylin levels positively correlate (data not shown), our findings would suggest that elevated circulating amylin levels may be related to the increased insulin secretion observed as a compensation for decreased insulin action; however, the exact form of amylin (reduced, unreduced, or amylin acid) which could be responsible remains elusive. Consistent with our proposal, amylin can act as a neuroendocrine hormone that complements the effects of insulin in postprandial regulation and there is evidence that in mice amylin improves leptin-mediated increases in insulin sensitivity [25]. As seen with our results, the unreduced (biological active) form failed to correlate with hypertriglyceridemia, indicating the mechanisms by which elevated levels of amylin may affect insulin action are unknown.

Studies on triglycerides and amylin in adult diabetics are scarce. However, infusion of amylin in rats lead to greater triglyceride content in liver and muscle tissue [10] and infusion of amylin-(8-37), a specific amylin antagonist, reduced muscle triglycerides concentration. On

	Total	Normal Glucose Tolerance	Impaired Glucose Tolerance
Sample (M/F)	140 (60/80)	81 (34/47)	59 (26/33)
Age (years)	41.6 ± 1.2	36.2 ± 1.3	48.9 ± 1.7*
Total Body Fat (%)	28.4 ± 0.7	27.2 ± 1.0	30.0 ± 1.1
Body Mass Index (kg/m ²)	26.2 ± 0.4	25.6 ± 0.5	27.0 ± 0.6
Systolic blood pressure (mmHg)	114 ± 1	113 ± 1	117 ± 1*
Diastolic blood pressure (mmHg)	75 ± 1	74 ± 1	76 ± 1
Fasting glucose (mg/dL)	95 ± 1	89 ± 1	102 ± 1*
Glucose 2-h postload (mg/dL)	110 ± 3	99 ± 2	127 ± 4*
Hemoglobin A1c (%)	5.03 ± 0.04	4.86 ± 0.05	5.21 ± 0.06*
Fasting insulin (μU/mL)	9.3 ± 0.4	8.7 ± 0.5	10.1 ± 0.7
Triglycerides (mg/dL)	155.7 ± 6.3	149.4 ± 8.4	164.2 ± 9.5
Total Amylin (pM)	7.6 ± 0.3	7.4 ± 0.4	7.8 ± 0.4
Reduced Amylin (pM)	8.1 ± 0.3	7.6 ± 0.4	8.7 ± 0.5

Table 1: Clinical and Metabolic characteristics Values are mean ± standard error. Significance was determined by Student T (Test. * p<0.05 Normal Glucose Tolerance vs. Impaired Glucose Tolerance).



the other hand, augmented plasma insulin enhanced muscle and liver insulin sensitivity and lowered plasma non-esterified fatty acids [15]. Studies in obese children showed that basal amylin levels were significantly correlated with insulin and triglycerides levels [6]. Interestingly, substantial weight loss in these children led to significant reduced amylin concentration that correlated with changes in insulin and triglycerides levels as well [8]. Percy et al. suggested that the different circulating forms of amylin (reduced and unreduced) or amylin-like molecules may have differential effects on the pathophysiology of Type 2 Diabetes [26]. Here, we determined that total amylin and not the unreduced form was associated with serum triglycerides. We posit that

the reduced amylin may have an unknown role in insulin secretion and consequently in Type 2 Diabetes disease development.

Our study has a few limitations. First, the study is a cross-sectional study and cannot determine any causal effect between any of the forms of amylin and hypertriglyceridemia. Second, it is important to note that our study only included Hispanic subjects thus providing valuable information on the relationship between amylin and triglycerides among an ethnically uniform group of subjects. However, further research among adult populations of different ethnic origins are needed to clarify these relationships. Third, our subjects were only first-degree

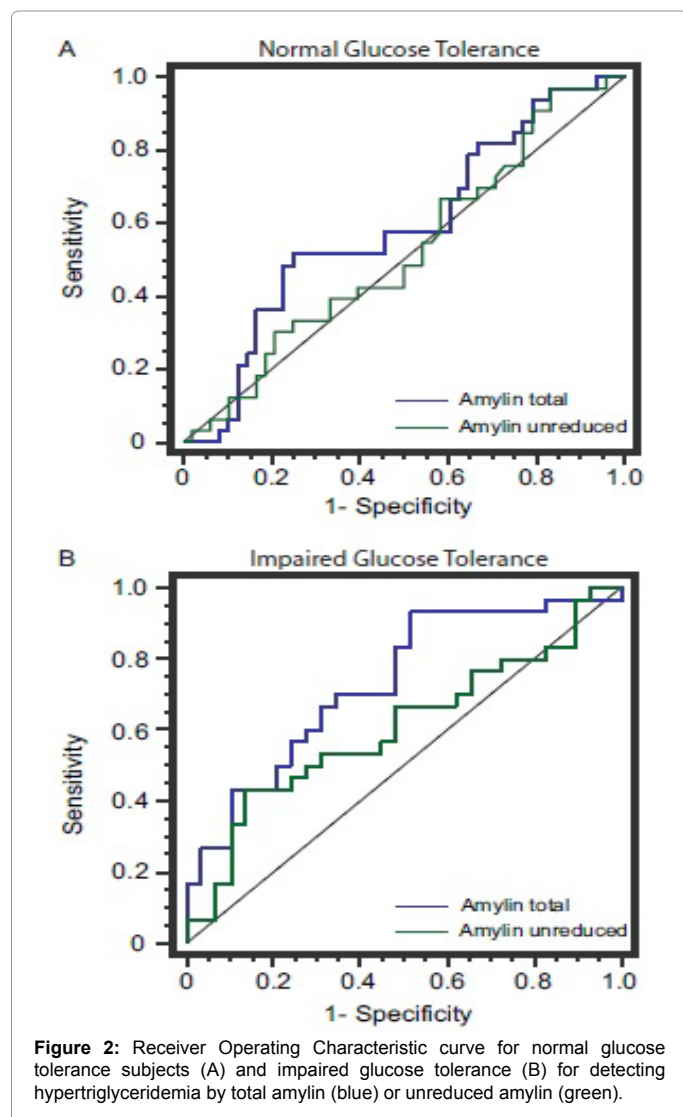


Figure 2: Receiver Operating Characteristic curve for normal glucose tolerance subjects (A) and impaired glucose tolerance (B) for detecting hypertriglyceridemia by total amylin (blue) or unreduced amylin (green).

relatives of Type 2 Diabetics. These results need to be confirmed among subjects without a genetics background for Type 2 Diabetes. Fourth, due to the size of the study, we were unable to determine the effects of obesity, hyperinsulinemia, or insulin resistance on amylin levels and hypertriglyceridemia.

In conclusion, our study suggests that serum triglycerides and total amylin levels are significantly associated in only pre-diabetics. Our study also supports the notion for examining reduced amylin and amylin-like molecules for their importance in Type 2 Diabetes development.

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