Acta Diabetol
DOI 10.1007/s00592-016-0904-7

ORIGINAL ARTICLE

Islet amyloid polypeptide response to maximal hyperglycemia and arginine is altered in impaired glucose tolerance and type 2 diabetes mellitus

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Received: 15 July 2016 / Accepted: 18 August 2016

Abstract
Aims Pancreatic islet amyloid deposition is a characteristic feature of type 2 diabetes mellitus (T2DM). Islet amyloid polypeptide (IAPP) is co-secreted with insulin, but its secretion profile and relationship to insulin and C-peptide in response to glucose and non-glucose stimuli has not been clearly defined.
Methods Forty subjects (13 NGT, 12 IGT and 15 T2DM) participated in an OGTT and two-step hyperglycemic (225 and 400 mg/dl) clamp (80 min/step) followed by an IV arginine bolus. Acute insulin (AIR), C-peptide (ACPR) and IAPP (AIAR) responses during each hyperglycemic step and following arginine (AIR_{Arg}) were assessed.
Results AIR and ACPR during both hyperglycemic steps and after arginine progressively decreased from NGT to IGT to T2DM. Fasting IAPP concentrations were higher in T2DM compared to NGT and IGT subjects. The acute $IAPP_{0–10}$ was markedly decreased only in T2DM, while the acute $IAPP_{80–90}$ response during the second step (80–160 min) of hyperglycemic clamp and in response to arginine was markedly impaired in both IGT and T2DM. The ratio of IAPP/C-peptide during the first (225 mg/dl) and second step (400 mg/dl), and in response to arginine, was decreased in T2DM versus both NGT and IGT ($p < 0.01$). The acute $IAPP_{0–10}$ correlated with $ACPR_{0–10}$ ($r = 0.665$, $p < 0.001$) and $AIR_{0–10}$ ($r = 0.543$, $p < 0.001$).
Conclusions Basal IAPP secretion is higher in T2DM and IGT versus NGT but is reduced in response to hyperglycemia and arginine. The IAPP/C-peptide ratio is reduced with prolonged and more severe hyperglycemia in T2DM individuals.
Clinical trial registration NCT00845182.

Keywords Insulin secretion · Amylin · Hyperglycemic clamp

Introduction

Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance in liver, muscle, adipose tissue and by progressive beta cell failure [1–4]. Islet amyloid polypeptide (IAPP) is co-secreted with insulin and represents the major constituent of pancreatic amyloid deposits in humans and non-human primates with T2DM [5–9]. Islet amyloid deposits have been associated with beta cell apoptosis, progressive loss of beta cell function and hyperglycemia in T2DM [10–20].

Earlier studies which examined IAPP secretion in T2DM provided varying results with regard to IAPP secretion. Fasting IAPP levels, as well as the IAPP/C-peptide ratio, have been reported to be reduced or normal in T2DM compared with NGT and IGT individuals [21–25]. There also have been conflicting reports concerning IAPP secretion and the IAPP/C-peptide and

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Published online: 13 September 2016

IAPP/insulin ratio during the OGTT and IVGTT, with both normal and reduced values being reported in T2DM [21–26]. Most of these earlier studies measured IAPP levels after an oral glucose load [21–23] and in few after an intravenous glucose stimulus [24–26]. Importantly, most earlier studies compared the IAPP response with plasma insulin levels, which are not the optimal parameter for assessing the beta cell function in insulin-resistant states, since up to 50 % of the insulin secreted by the pancreas is cleared by the liver [27]. Also, in these previous studies IAPP secretion was not related to insulin sensitivity to assess the gold standard measure of beta cell function (Δ insulin secretion/Δ glucose $\times$ insulin resistance). In the present study, we characterize IAPP secretion in NGT, IGT and T2DM individuals during OGTT and during a two-step hyperglycemic clamps followed by intravenous arginine to identify abnormalities in IAPP secretion in IGT and T2DM.

Materials and methods

Experimental protocol

The study protocol was approved by the Institutional Review Board of University of Texas Health Science, and all subjects provided signed voluntary informed consent. Fifteen patients with T2DM, 12 with IGT and 13 healthy control subjects received a 2-h oral glucose tolerance test on the Bartter Research Unit (BRU) at the South Texas Veterans Health Care System, following a 10-h overnight fast. Plasma glucose, insulin and C-peptide were measured at -30, -15, and 0 min and every 15 min thereafter for 2 hours. IAPP concentrations were measured at 0, 30, 60 and 120 min. All study participants had normal renal function (eGFR>60 ml/kg/min); of the 15 T2DM patients, ten were on combination of metformin and glyburide, four were on metformin only and one was only on diet. Healthy controls and IGT subjects were not on any medication. All anti-diabetic medications were withheld 24 h before the OGTT and hyperglycemic clamp study.

Two-step hyperglycemic clamp

Following an overnight fast, subjects were admitted to the Bartter Research Unit (BRU) at 6:30 AM the day of the study. A low-dose insulin (0.1–0.3 mU/kg/min) infusion was started in individuals with T2DM to reduce the fasting plasma glucose concentration to $\sim$100 mg/dl. The insulin infusion was discontinued 30 min prior to the hyperglycemic clamp. At time zero, the plasma glucose concentration was acutely raised and maintained at 125 mg/dl above baseline (i.e., approximately 225 mg/dl) for 80 min. Plasma glucose was measured every 2 min during the first 10 min and every 5 min for the subsequent 70 min. At 80 min, the plasma glucose concentration was acutely raised and maintained at 400 mg/dl for an additional 110 min. Plasma glucose was measured every 2 min from 80 to 90 min and every 5 min from 90 to 160 min. This provided a maximum hyperglycemic stimulus to insulin secretion. At 160 min, an intravenous bolus of 5 g of arginine was given, and the plasma glucose was measured every 2 min for 10 min following the arginine bolus (160–170 min) and every 5 min for the subsequent 20 min. The combination of hyperglycemia plus hyperaminoacidemia provides a maximal stimulus for insulin secretion [28]. IAPP, insulin and C-peptide levels were measured at 0, 2, 6, 10, 80, 160, 162, 166, 170 and 190 min during the two-step hyperglycemic/arginine clamp.

Analytical measurements

Plasma glucose concentration was determined by the glucose oxidase reaction (Glucose Oxidase Analyzer; Beckman, Fullerton, CA), and plasma insulin and C-peptide concentrations were measured by radioimmunoassay (Coat A Coat; Diagnostic Products, Los Angeles, CA). Total IAPP was measured with ELISA (LINCO Research, St.Charles, MO, USA).

Statistical analysis and calculations

Data are presented as mean $\pm$ SEM. Incremental AUC (ΔAUC) for glucose, insulin, C-peptide and IAPP was calculated using the trapezoidal rule. Whole body insulin sensitivity during the OGTT was calculated with the Matsuda index as previously described [29]. Insulin secretion was calculated as Δinsulin/Δglucose for specific time intervals (0–10 and 10–80 min). Beta cell function was calculated as $\Delta I/\Delta G \times$ insulin sensitivity and $\Delta CP/\Delta G \times$ insulin sensitivity. Comparison of numerical variables between groups was done with the analysis of variance (ANOVA), and a Bonferroni post hoc analysis. Correlation analysis was done with the Pearson correlation coefficient. In order to adjust for the confounding variables, we performed a paired analysis by age and BMI, including seven patients per group. p values <0.05 were considered statistically significant.

Results

Clinical and metabolic characteristics

Table 1 describes the clinical features. Individuals with IGT and T2DM were slightly older and had a higher BMI than

Table 1 Clinical and laboratory characteristics of the study population

Variable	NGT $n = 13$	IGT $n = 12$	T2DM $n = 15$	p value
Gender (M/F)	5/8	5/7	12/3	0.032
Age (years)	41.0 ± 3.7	52.1 ± 2.9[a]	56.2 ± 1.7[a]	0.001
T2DM duration (years)	–	–	6.4 ± 4.1	–
Weight (kg)	77.8 ± 8.0	85.3 ± 3.7	100.1 ± 4.6[a]	0.022
BMI (kg/m^2)	26.4 ± 2.2	30.9 ± 1.2	31.6 ± 1.3[a]	0.003
FPG (mg/dl)	93 ± 2	105 ± 2	172 ± 10[a b]	<0.001
FPI (μIU/l)	3.7 ± 1.2	6.1 ± 1.2	16.8 ± 5.7[a]	0.049
C-peptide (pmol/l)	888 ± 165	1212 ± 152	1255 ± 164	0.154
2 h PG (mg/dl)	99 ± 4	158 ± 5[a]	296 ± 15[a b]	<0.001
HbA1c (%)	5.2 ± 0.1	5.8 ± 0.1[a]	8.2 ± 0.2[a b]	<0.001
TC (mg/dl)	168 ± 6	173 ± 14	167 ± 9	0.892
LDL (mg/dl)	103 ± 8	110 ± 12	108 ± 10	0.897
HDL (mg/dl)	47 ± 4	38 ± 4	35 ± 1.3[a]	0.013
TG (mg/dl)	87 ± 13	199 ± 66	188 ± 37	0.110
Insulin sensitivity index	13.6 ± 1.9	4.9 ± 0.8[a]	2.7 ± 0.3[a b]	<0.001
Insulin secretion/insulin resistance index (0–10 min)	3622 ± 669	1555 ± 474[a]	38 ± 19[a b]	<0.001

[a] Versus Group 1

[b] Versus Group 2

NGT subjects, although there were no differences in age and BMI between IGT and T2DM. Fasting and 2-h plasma glucose, fasting and 2-h plasma insulin and C-peptide, and HbA_{1c} increased progressively from NGT to IGT to T2DM. There was a progressive and marked reduction in insulin sensitivity and beta cell function (Table 1, $p < 0.001$) with worsening of glucose tolerance from NGT to IGT to T2DM. Of the patients with T2DM, ten were treated with a combination of metformin and glyburide, four were on metformin only and one was on dietary treatment only.

Plasma IAPP concentrations

The fasting IAPP concentration was 8.7 ± 1.6, 12.7 ± 1.7 and 15.1 ± 1.9 pmol/l in NGT, IGT and T2DM, respectively ($p < 0.001$, NGT vs T2DM; Fig. 1a); the fasting IAPP/C-peptide ratio, 2 h postglucose IAPP concentrations and 2 h postglucose IAPP/C-peptide ratio were not statistically different between groups (Fig. 1b–d, p = NS). The incremental area under the IAPP (ΔAUC_IAPP$_{0–120}$) and C-peptide (ΔAUC_CP$_{0–120}$) curve during the OGTT was lower only in T2DM (Fig. 2a, b, $p < 0.001$). However, when adjusted for glucose levels, the ΔAUC_IAPP/ΔAUC_Glucose$_{0–120}$ and ΔAUC_C-peptide/ΔAUC_Glucose$_{0–120}$ progressively declined from NGT to IGT to T2DM (Fig. 2c, d $p < 0.001$). The ΔAUC_IAPP$_{0–120}$ correlated with the ΔAUC_C-peptide$_{0–120}$ during OGTT ($r = 0.574$, $p = 0.01$). The difference between the groups persisted after the paired analysis and adjustment for the difference in age and BMI ($p < 0.01$).

Plasma IAPP concentration during hyperglycemic clamp

The ΔAUC_IAPP$_{0–10}$ during the hyperglycemic clamp was reduced in T2DM compared to NGT (Fig. 3a, $p < 0.001$). The acute IAPP response to arginine (ΔAUC_IAPP$_{160–170}$) also was reduced in T2DM compared to NGT and IGT (Fig. 3b, $p < 0.001$). The incremental insulin response (Δ_AUC) was reduced in T2DM both in response to hyperglycemia (0–10 min) (Fig. 3c, $p < 0.001$) and in response to arginine (160–170 min; Fig. 3d, $p < 0.001$). During the first step of the hyperglycemic clamp (0–80 min), IGT individuals had higher IAPP values than T2DM patients, and during the second hyperglycemic clamp step (80–160 min) the IAPP values were lower in T2DM than in NGT and IGT (Fig. 4a, $p < 0.05$). The plasma C-peptide response was decreased in T2DM during both hyperglycemic steps and in response to arginine (Fig. 4b, $p < 0.05$). IAPP/C-peptide ratio was not statistically different between groups during the first hyperglycemic clamp step (Fig. 4c, p = NS). However, during the second hyperglycemic clamp step the patients with type 2 diabetes had reduced IAPP/C-peptide ratio versus NGT and IGT subjects and declined further after arginine (Fig. 4c, $p < 0.05$). The difference between the groups was more pronounced after adjustment for age and BMI ($p < 0.01$). As expected, ΔAUC_IAPP$_{0–10}$ and ΔAUC_IAPP$_{160–166}$ were progressively reduced in IGT and T2DM versus NGT subjects when adjusted for the prevailing insulin sensitivity.

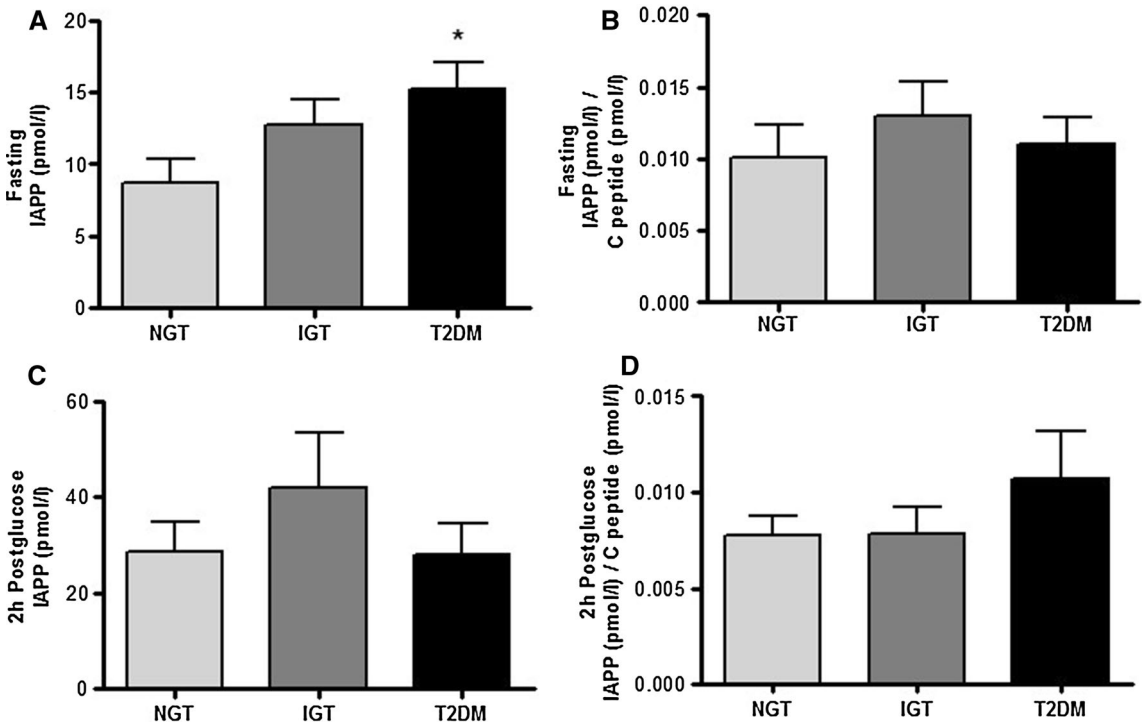

Fig. 1 Fasting IAPP (**a**) and IAPP/C-peptide ratio (**b**), and 2 h post OGTT IAPP (**c**) and IAPP/C-peptide ratio (**d**) in patients with NGT, IGT and T2DM. *$p < 0.05$ versus NGT

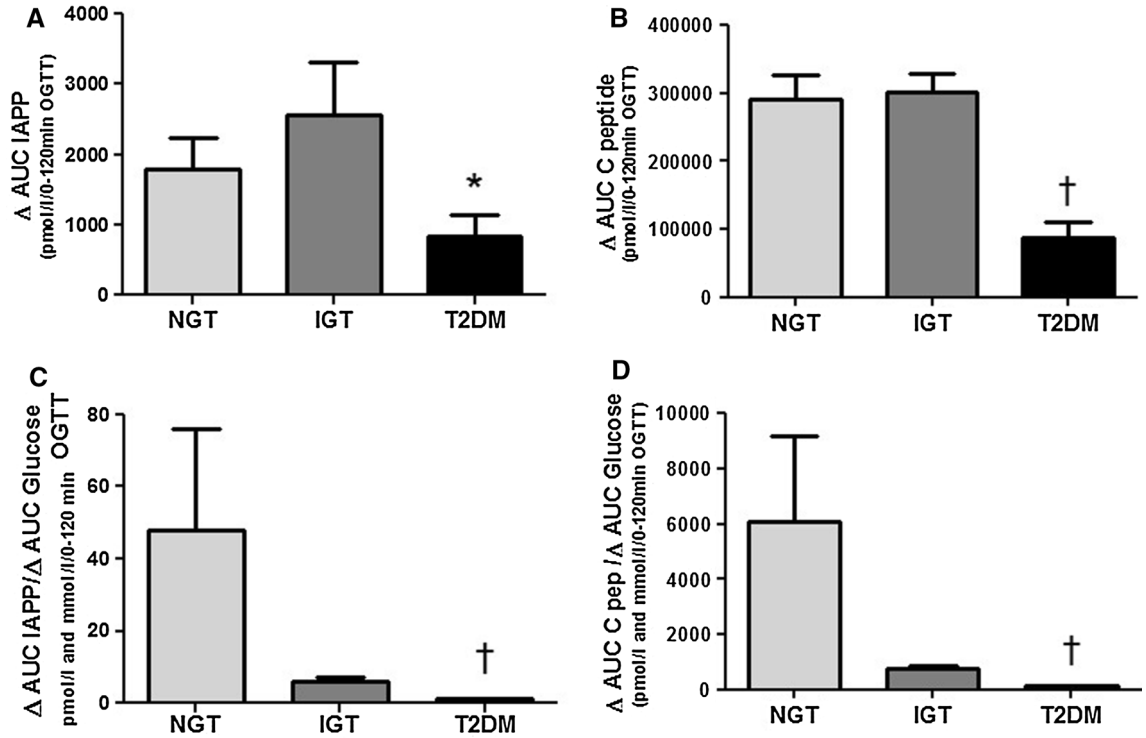

Fig. 2 Incremental area under the IAPP (**a**) and C-peptide (**b**) curve during the OGTT, and adjusted by the Incremental area under the glucose curve (**c**, **d**). *$p < 0.001$ versus IGT, †$p < 0.001$ versus NGT and IGT

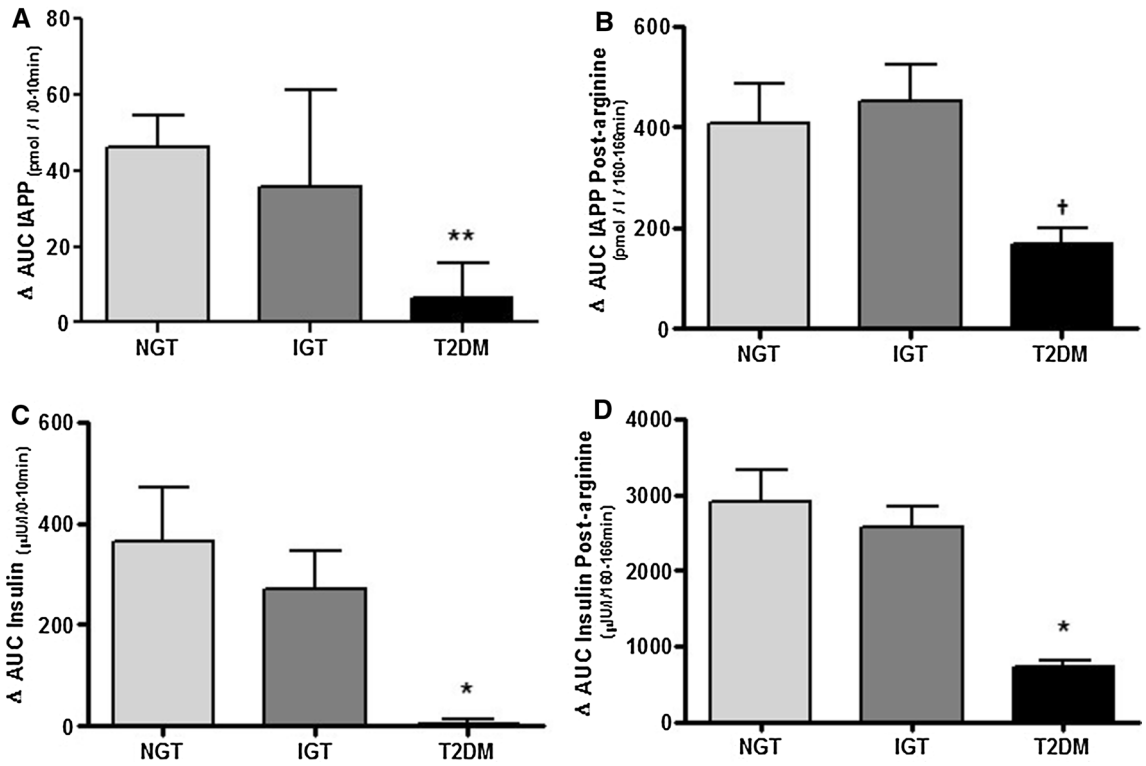

Fig. 3 Incremental AUC for IAPP and insulin during the two-step hyperglycemic clamp. **a** IncAUC $IAPP_{0–10\ min}$, **b** IncAUC $IAPP_{160–166\ min}$, **c** IncAUC $insulin_{0–10\ min}$ and **d** IncAUC $insulin_{160–166\ min}$. $^{*}p < 0.001$ versus NGT, $^{**}p < 0.05$ versus IGT, $^{\dagger}p < 0.05$ versus NGT and IGT groups

Relationship between plasma IAPP, insulin and C-peptide concentrations during hyperglycemic clamp

Since insulin, C-peptide and IAPP are co-secreted in equimolar amounts, one would expect a positive relationship between these hormones during the OGTT and during the hyperglycemic clamp. Fasting plasma IAPP levels correlated with fasting insulin (Fig. 5a, $r = 0.640$, $p < 0.001$) and C-peptide (Fig. 5b, $r = 0.666$, $p < 0.001$), and this correlation remained significant after adjustment by group and the paired analysis by age and BMI ($p < 0.001$). As expected, $\Delta AUC\_IAPP_{0–10}$ correlated better with the $\Delta AUC\_C\text{-}peptide_{0–10}$ (Fig. 5c, $r = 0.701$, $p < 0.001$) than with the $\Delta AUC\_insulin_{0–10}$ (Fig. 5d, $r = 0.541$, $p < 0.001$). The correlation between the $\Delta AUC\_IAPP_{0–10}$ and the $\Delta AUC\_C\text{-}peptide_{0–10}$ was even stronger in IGT patients ($r = 0.798$, $p < 0.001$).

Discussion

In the present study, we have examined the dynamic change in IAPP secretion in individuals with NGT, IGT and T2DM. The novelty of this study is the characterization of IAPP secretion in relation to C-peptide secretion in response to maximal hyperglycemia and a non-glucose stimulus arginine. Abnormalities in IAPP secretion/production could play an important role in the rate of islet amyloid deposition and, therefore, in the decline in beta cell function and pathogenesis of T2DM since degradation products of IAPP are toxic to the beta cell and precursors of IAPP are more prone to precipitate [10–17, 19, 30–38]. Previous studies have reported reduced or normal IAPP levels in T2DM in response to glucose ingestion [21–23]. In the present study, we show that absolute fasting IAPP levels are increased in T2DM compared to both NGT and IGT subjects. Since IAPP is co-secreted along with insulin [39, 40] and since basal insulin secretion is increased in T2DM [41], this is not surprising. The fasting plasma IAPP/C-peptide ratio has been reported to be reduced or normal in T2DM [23, 24]. In the present study, we found a slight, albeit nonsignificant increase in this ratio. In agreement with previous studies, we also found slight reduction in IAPP levels in T2DM, which is similar to or slightly increased when expressed by C-peptide (IAPP/C-peptide ratio) during the OGTT (Suppl. Fig. 2a–c) [21–23]. This is as expected since IAPP and C-peptide are co-secreted and neither are extracted by the liver.

Most previous studies have measured IAPP levels during fasting conditions or after an oral glucose load, and few

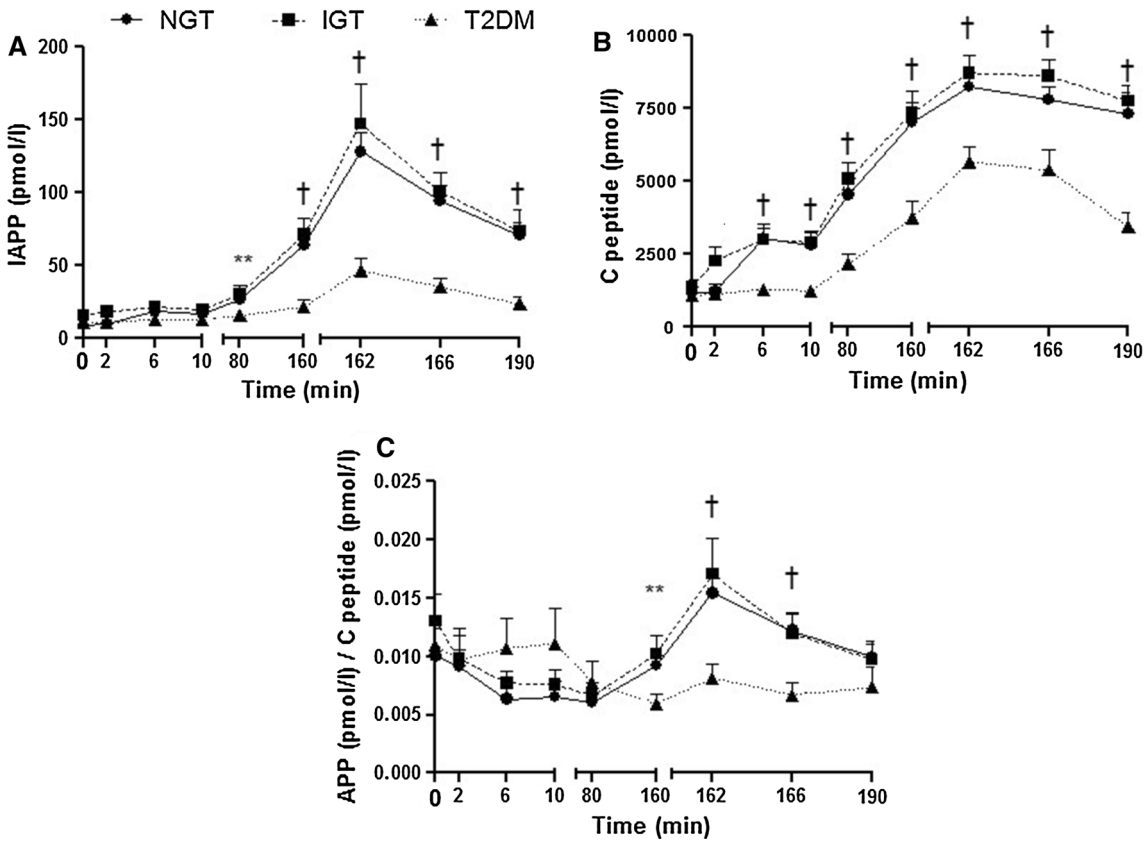

Fig. 4 IAPP (**a**), C-peptide (**b**) and IAPP/C-peptide ratio (**c**) during the two-step hyperglycemic clamp. $^{**}p < 0.05$ for comparisons IGT versus T2DM, $^{\dagger}p < 0.05$ for comparisons T2DM versus NGT and IGT

studies have examined the IAPP response to IV glucose or arginine. During the IVGTT, the administration of bolus of glucose leads to varying glucose levels and, thus, a varying stimulus to the beta cell for insulin/IAPP secretion. Further, and most importantly, most previous studies have related the plasma IAPP levels to the plasma insulin levels, which is not the optimal parameter to assess beta cell secretory capacity, since up to 50 % of the insulin secreted by the pancreas is cleared by the liver [27]. After an IV glucose bolus and during the IVGTT, both the IAPP/C-peptide and IAPP/insulin ratio have been reported to be similar in T2DM and NGT groups [24, 25]. During a 2-h hyperglycemic clamp, both the first and second phase of IAPP secretion have been reported to be reduced in T2DM, while in IGT only the first phase of IAPP secretion was decreased compared with NGT subjects [26]. In the present study, first phase IAPP secretion also was reduced in T2DM versus IGT subjects, and the IAPP response to arginine was reduced in T2DM versus IGT subjects. Thus, in T2DM subjects the acute IAPP response to both glucose and amino acids is reduced.

In freshly isolated human islets, the basal IAPP/insulin molar ratio in response to 5.5 and 16.7 mM glucose was 1:16 and 1:15, respectively [42]. When the islets were cultured for 1 and 7 days, at 5.5 and 16.7 mM glucose, the IAPP/insulin molar ratio was similar at the first day, but, after long-term exposure to hyperglycemia (7 days at 16.7 mM glucose), IAPP and insulin secretory response were dissociated. While insulin secretion was significantly increased, the IAPP secretion was reduced leading to a reduced IAPP/insulin ratio. Additionally, the IAPP content was higher than insulin content after long exposure of islets to high glucose concentrations even though the increase was significant for both peptides. Northern blot analysis during each cultured condition showed an increase in both IAPP and insulin mRNA following exposure of islets to 16.7 mM glucose, suggesting that IAPP secretion by the beta cells is significantly reduced compared to insulin secretion after prolonged exposure of human islets to high glucose concentrations, even though IAPP synthesis may not be affected, which could lead to an IAPP accumulation into the beta cell [42]. In agreement with this, in the present study, the IAPP/C-peptide ratio during the first 10 min of the hyperglycemic clamp was higher in T2DM versus NGT subjects, even though the ratio declined progressively from NGT to IGT to T2DM. However, after prolonged and more severe hyperglycemia ($\geq$80 min during the second hyperglycemic clamp step) the IAPP/C-peptide ratio decreased

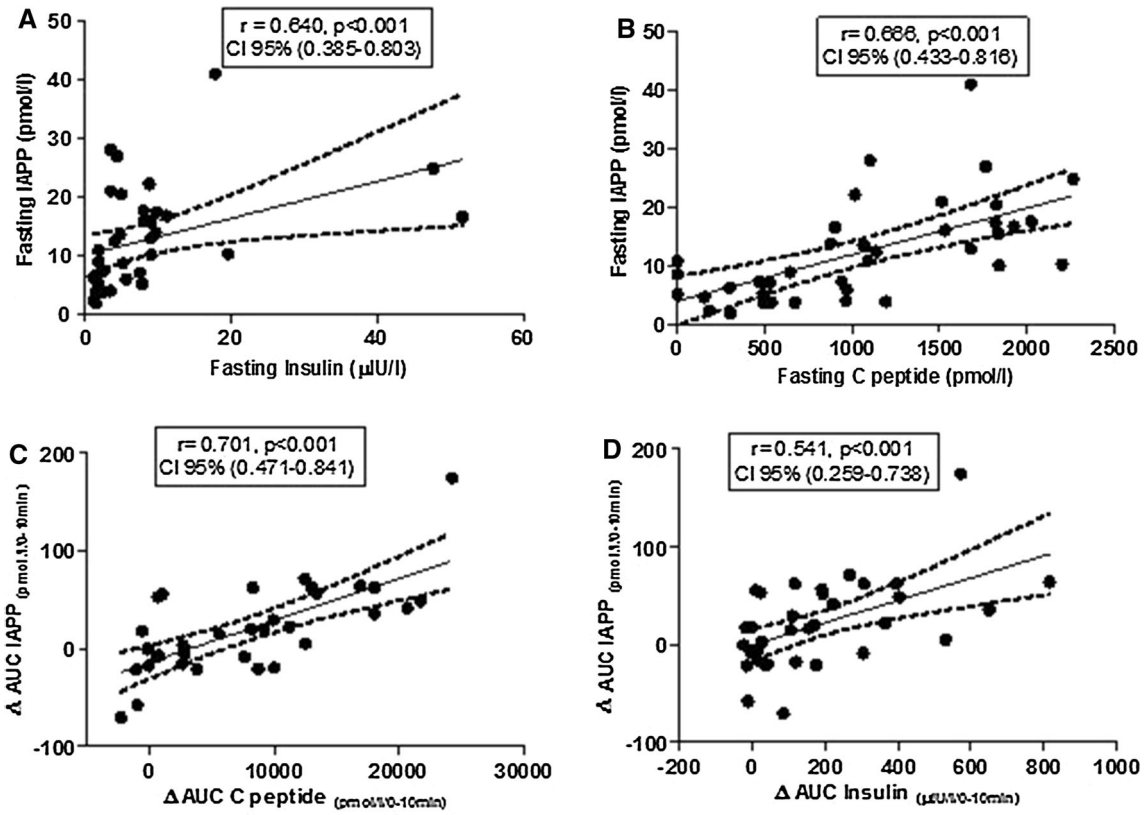

Fig. 5 Correlation between fasting IAPP with fasting insulin (**a**) and C-peptide (**b**); correlation between Incremental AUC IAPP with the incremental AUC C-peptide from 0 to 10 min (**c**) and with the incremental AUC insulin 0–10 min (**d**)

markedly in T2DM compared with NGT and IGT subjects. This decrease was more marked when the beta cell was exposed to a maximal hyperglycemic stress plus arginine. To our knowledge, this is the first study in vivo in humans to document a reduction in the IAPP/C-peptide ratio in response to a progressively increasing beta cell stress, and our results are in agreement with an in vitro study performed in cultured human islets [42]. Of note, C-peptide and IAPP clearance are factors that potentially could affect these results. C-peptide clearance has been reported to be slightly higher in obese subjects, due to the higher distribution volume, and not different in T2DM when compared to normal glucose tolerant subjects without renal impairment [43]. In one study, IAPP clearance was measured and was found to be similar to C-peptide clearance in control subjects, though renal failure may reduce IAPP clearance since about 70 % of the IAPP is cleared by the kidney [44]; since all subjects included in our study had normal renal function, it is unlikely to affect the results. Besides that, the differences in IAPP levels and IAPP/C-peptide ratio persisted even after the adjustment and paired analysis by age and BMI ($p < 0.01$).

Our results, in combination with other reports [35, 38, 39], suggest that abnormalities in the IAPP processing could be an important factor in the progression of glucose intolerance and pancreatic islet dysfunction as one progress from NGT to IGT to T2DM. Of note, ten patients with T2DM were on a sulphonylurea, glyburide, but it was withheld at least 24 h before the OGTT and two-step hyperglycemic clamp, and thus should not influence IAPP secretion.

Whether these abnormalities in the mechanisms of IAPP processing and secretion are also involved in the progressive loss of beta cell function observed after islet transplantation in humans with diabetes, remains unclear and is currently being examined. Previous studies have shown alterations in proinsulin processing as well as amyloid deposition in patients with short-term functioning graft, and these abnormalities could affect the beta cell—islet function, possibly also in combination with the immunosuppressive therapies that these patients receive [45–48]. The present study has some limitations. First, the number of subjects in each group is relatively small. However, this is compensated by use of the hyperglycemic clamp, which is the gold standard for the estimation of insulin secretion, is highly reproducible and allows one to present a constant and similar hyperglycemic stimulus to the beta cell in all groups, which would be expected to reduce variability.

Second, both the IGT and T2DM subjects were slightly older and more obese than the NGT group. However, there were no significant differences in either age or BMI between the IGT and T2DM groups, and a paired analysis after adjusting for age and BMI showed the same trend and statistical significance. Therefore, the marked decline in insulin, C-peptide and IAPP secretion in T2DM versus IGT subjects is unlikely to be explained by the modest differences in age and BMI. To the contrary, the plasma insulin and C-peptide responses to glucose and arginine in the NGT group were further enhanced after the NGT group was matched for obesity, since obesity is an insulin-resistant state associated with a compensatory increase in insulin secretion.

In conclusion, basal IAPP secretion is higher in T2DM and IGT versus NGT but is reduced in response to hyperglycemia and arginine, particularly when adjusted for insulin sensitivity. The IAPP/C-peptide ratio is reduced with prolonged and more severe hyperglycemia in T2DM individuals, which could be a marker of abnormal IAPP processing and secretion during β cell stress. This potentially could result in pancreatic amyloid deposition and contribute to the progressive beta cell dysfunction that is a characteristic of T2DM.

Acknowledgments We thank the nurses on the Bartter Research Unit (BRU) for their diligent care of our patients and especially James King, R.N. and John Kincade RN for carrying out the hyperglycemic clamp studies. The study was supported by a research grant from Takeda Pharmaceuticals to Devjit Tripathy.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interests.

Human and animal rights disclosure All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5).

Informed consent disclosure Informed consent was obtained from all patients for being included in the study.

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