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Incretin release and aging

Older Subjects with β-cell Dysfunction have an Accentuated Incretin Release

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Objective: Insulin secretion declines with age and this contributes to the increased risk of developing impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM) in older subjects. Insulin secretion is regulated by the incretin hormones glucagon-like peptide (GLP) 1 and glucose-dependent insulinotropic peptide (GIP). Here we tested the hypotheses that incretin release is reduced in older subjects, and that this decline is associated with β-cell dysfunction.

Research Design: 40 young (25±3 y) and 53 older (74±7 y) lean non-diabetic subjects underwent a 2 h oral glucose tolerance test (OGTT). Based on the OGTT, subjects were divided in 3 groups: young normal glucose tolerant (Y-NGT, n=40), older with NGT (O-NGT, n=32), and older with IGT (O-IGT, n=21).

Main Outcome Measures. Plasma insulin, C-peptide, GLP-1, and GIP concentrations were measured every 15-30 min. We quantitated insulin sensitivity (Matsuda index) and insulin secretory rate (ISR) by deconvolution of C-peptide with the calculation of β-cell glucose sensitivity.

Results. Matsuda index, early phase ISR (0-30min) and parameters of β-cell function were reduced in O-IGT vs. Y-NGT, but not in O-NGT. GLP-1 concentrations were elevated in both older groups [GLP-1_AUC_{0-120} was 2.8±0.1 in Y-NGT, 3.8±0.5 in O-NGT, and 3.7±0.4 nmol/l·120 min in O-IGT ($P<0.05$)] while GIP secretion was elevated in O-NGT vs. Y-NGT [GIP_AUC_{0-120} was 4.7±0.3 in Y-NGT, 6.0±0.4 in O-NGT, and 4.8±0.3 nmol/l·120 min in O-IGT ($P<0.05$)].

Conclusions: Aging is associated with an exaggerated GLP-1 secretory response. However, this was not sufficient to increase insulin first phase release in O-IGT and overcome insulin resistance.

Aging is associated with an exaggerated GLP-1 secretory response. However, this was not sufficient to increase insulin release and overcome insulin resistance in older subjects with IGT.

INTRODUCTION

Aging is associated with major changes in glucose metabolism. Various studies indicate that increasing age is accompanied by impaired glucose tolerance. The 2 hour plasma glucose concentration during an oral glucose tolerance test (OGTT) rises on average 5.3 mg/dl per decade (1). The Baltimore Longitudinal Study of Aging showed a progressive decline in glucose tolerance from the third through the ninth decade of life (2). This decline in glucose tolerance with age also was evident in the National Health and Nutrition Examination Survey (NHANES) III, in agreement with recent estimates that approximately one third of subjects aged ≥65 have diabetes (3). The cause for the high prevalence of glucose intolerance and type 2 diabetes mellitus (T2DM) in the older population is not clear.

Yet, many factors have been implicated, including changes in fat distribution (4), physical activity (5), muscle insulin sensitivity (6), and β cell function (7).

A decrease in β cell function with advancing age has been previously documented (8). For example, β cell responsiveness during a frequent sampled intravenous glucose tolerance test is reduced in older nondiabetic subjects compared with younger individuals (9,10). In addition, β cell insulin response upon arginine stimulation also is impaired in older subjects (9,10). Despite these data indicating that aging leads to decreases in β cell function, the cause for this age-dependent functional decline is not known. Some studies have found that β cell mass declines with age (11), although other studies have not reported such decline (12).

incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) peptides are secreted by the gut in response to nutrients, enhancing insulin secretion by β cells and reducing glucagon release (14). While T2DM and adiposity are associated with altered incretin release and β cell resistance to both GLP-1 and GIP (15,16), t is still unclear whether the decline in β cell function seen in normal aging (i.e., in the absence of T2DM) also is associated with defects in incretin secretion.

The purpose of this study was to evaluate incretin hormone secretion in lean older subjects, with either normal (NGT) or impaired (IGT) glucose tolerance, compared to lean young NGT. We also examined whether possible age-related differences in incretin release are associated with changes in β cell function. Because incretins enhance β cell function and mass (15), we hypothesized that aging leads to a reduced and/or impaired incretin release in response to a glucose load, and that this impairment would be associated with reduced β cell function.

METHODS

Subjects.

We studied 40 young (18-30 years old) and 53 older (≥ 65 years old) non-diabetic non-obese subjects. Each subject underwent a medical history, physical examination, screening laboratory tests, and a 75-g oral glucose tolerance test (OGTT). All subjects were sedentary (not more than one session of exercise per week) and community-dwelling. Subjects were not obese (BMI = 23-26 kg/m^2) and did not have a family history (first degree relative) of diabetes. Body weight was stable (± 1 kg) for at least 3 months prior to enrollment. Subjects were not taking medication known to affect glucose metabolism. The study was approved by the Institutional Review Board of the UTHSCSA and all subjects gave written voluntary consent.

OGTT.

Plasma glucose, insulin, and C peptide concentrations were measured at baseline and every 15 min for 2 h after the ingestion of 75 g glucose; GLP-1 and GIP were measured every 30 minutes in samples collected in prechilled test tubes containing aprotinin and EDTA. Based on the OGTT, subjects from the older group were subdivided into NGT or IGT groups. All subjects in the young group were NGT. The incremental AUC for plasma glucose and insulin during the OGTT was calculated using the trapezoidal rule (17).

We calculated the HOMA insulin resistance (IR) index and the Matsuda index for insulin sensitivity, as previously described (18,19). The primary stimulus for insulin secretion is the increment in plasma glucose in the first minutes after the glucose load. Thus, we calculated the insulinogenic index as the incremental area AUC for plasma insulin concentration (ΔI) divided by the incremental AUC for plasma glucose concentration (ΔG) from 0 to 30 min and the late insulin response as ΔI/ΔG from 30 to 120 min. The pre-hepatic insulin secretory rate (ISR) was calculated by plasma C- peptide deconvolution (20,21). We also calculated the

indexes of β-cell insulin secretion, the rate sensitivity (Kd), i.e., the insulin secretion in response to changes in glucose concentration, and the β-cell glucose sensitivity, i.e., the slope of the dose response between ISR and glucose excursion, as previously described (22,23). The disposition index during OGTT was calculated as ΔI/ΔG× Matsuda index from 0-30 and 0-120 minutes, respectively (21,24). We also calculated the insulin secretion/insulin resistance (IS/IR) index as ΔISR/ΔG × Matsuda index (21,24).

Laboratory analyses.
Plasma insulin and C-peptide concentrations were measured by radioimmunoassay (Diagnostic Products, Los Angeles, CA), glucose was measured with the oxidase method on a Beckman Analyzer, and hemoglobin A1c was measured using a DCA 2000 analyzer (Bayer Corporation, Tarrytown, NY). GIP was measured by radioimmunoassay with a C-terminally directed antiserum code # 867, raised against a synthetic peptide corresponding to the C-terminus of human GIP (University of Copenhagen, Denmark) thus measuring "total" GIP (intact GIP + the primary metabolite GIP 3-42). Total GLP-1 (intact GLP-1 + the primary metabolite GLP-1 9-36 amide) was measured by radioimmunoassay using standards of synthetic GLP-1 7-36 amide and antiserum code no. 89390, which is specific for the amidated C-terminus of GLP-1 (University of Copenhagen, Denmark). Plasma concentrations of total cholesterol and triglyceride were measured enzymatically (Boehringer-Mannheim, Indianapolis, IN). Plasma HDL cholesterol was measured enzymatically on Hitachi 704 autoanalyzer after precipitation of chylomicron and VLDL and LDL cholesterol by phosphotungstic acid. LDL cholesterol was calculated from the Friedwald equation.

Statistical methods.
mean ± standard error and qualitative variables were expressed as percentages. Kolmogorov-Smirnov test was performed to evaluate distribution of the variables. Comparison between groups (Young vs Old) was performed using t-test for quantitative variables with normal distribution and with Mann Whitney U test for those with non-normal distribution. To compare more than two groups we used ANOVA and Tukey's test for normally distributed variables and Kruskal-Wallis's procedure for non-normal data distribution. Correlations between continuous variables were carried out using Pearson correlation for variables with normal distribution and Spearman for those with non normal distribution.

RESULTS

Subjects' Characteristics.
We studied 93 subjects sub-divided in 3 groups according to age and degree of glucose tolerance: young subjects with NGT (Y-NGT; n=40, mean age 25 y), older subjects with NGT (O-NGT; n=32, mean age 72), and older subjects with IGT (O-IGT; n=21, mean age 77). Anthropometric and metabolic characteristics of the subjects are shown in **Table 1**. Sex distribution and BMI were not statistically different between the three groups. Total and LDL cholesterol concentrations were higher in older IGT compared with NGT subjects (both Y-NGT and O-NGT), while the HDL cholesterol and triglycerides were similar in all groups. The fasting plasma insulin was similar in the 3 groups while the fasting plasma glucose was slightly but significantly elevated in both older groups (O-NGT, O-IGT) compared with the Y-NGT group (**Table 1**). The glucose AUC increased progressively from Y-NGT to O-NGT to O-IGT (**Figure 1A**).

Indexes of insulin sensitivity.
The HOMA IR, which primarily represents hepatic insulin sensitivity, was not significantly different between the three groups (**Table 2**). Peripheral insulin sensitivity, calculated with the Matsuda index, was similar among NGT subjects (i.e., O-NGT and Y-NGT) but was

significantly reduced in the older IGT group (P<0.05 vs. Y-NGT) (**Table 2**). ΔG_{0-120} was significantly elevated in the O-IGT group, compared with both Y-NGT and O-NGT. The O-IGT group displayed a significant increase in glucose concentrations particularly from 30-120min (**Table 2**, **Figure 1A**).

Insulin response to a glucose load.

Plasma glucose concentrations were similar in all subjects in the first 30 minutes of the OGTT (**Figure 1A**); glucose concentrations of O-NGT overlapped those in Y-NGT, while in O-IGT glucose concentrations were higher (**Figure 1A**). The early incremental plasma insulin response (both ΔI_{0-30} and ΔISR_{0-30}) to the OGTT was reduced only in O-IGT vs. Y-NGT (**Figure 1 panel B and C**) while insulin secretion from 30-120min (ΔI_{30-120} and ΔISR_{30-120}) was increased in the O-IGT (**Table 2**). Since older patients had a late response in insulin secretion both ΔI_{0-120} and ΔISR_{0-120} were similar in the 3 groups (**Table 2).**

β cell function.

We have calculated the rate sensitivity (Kd), i.e., the insulin secretion in response to changes in glucose concentration and the β-cell glucose sensitivity, i.e., the slope of the dose response between ISR and glucose excursion as previously described. The β-cell glucose sensitivity was reduced only in the O-IGT. Also the disposition index and the insulin secretion/insulin resistance ratio were reduced only in the O-IGT, both when calculated in the first 30 min or during the entire duration of the OGTT (**Table 2**).

Incretin secretion.

GLP-1 secretion was significantly increased in both older groups vs. Y-NGT [GLP-1_AUC_{0-120} was 2.8±0.1 in Y-NGT, 3.8±0.5 in O-NGT, and 3.7±0.4 nmol/l·120 min in O-IGT (P<0.05)]. There was no difference in GLP-1 secretion between older groups (**Figure 2**A). GIP secretion was significantly elevated in the O-NGT group [AUC_{0-120} 6.0±0.4 nmol/l x 120min) compared to Y-NGT and to O-IGT (4.7±0.3 and 4.8±0.3 nmol/l x 120min] (**Figure 2B**). Even though the main effect of aging on β cell function is most evident during the first 30 minutes of the OGTT, the GLP-1_AUC during the last 60 min of the curve remained significant elevated in O-NGT compared with Y-NGT subjects, suggesting a compensatory response of GLP-1 secretion in O-NGT. We analyzed the relationship between incretin secretion and insulin response by plotting the incremental incretin concentrations during the first 60 min vs. the incremental ISR. We observed that O-IGT had increased GLP-1 secretion compared to Y-NGT but significantly lower ISR compared to both Y-NGT and O-NGT (**Figure 2C**). O-NGT and Y-NGT had similar ISR response in the first hour but GIP secretion was higher in O-NGT, perhaps indicating lower sensitivity to GIP in older subjects (**Figure 2D**). On the other hand, O-IGT had reduced both GIP and ISR during the first 60 min of the OGTT compared to O-NGT (**Figure 2D**).

DISCUSSION

We investigated the relationship between age-related changes in β cell function with incretin secretion in response to oral glucose. The loss of first phase insulin secretion (1^{st} phase ISR) is one of the earliest abnormalities observed in glucose intolerant individual (25-27). Chen et al demonstrated that older subjects may loose 1^{st} phase ISR and have a delayed insulin response in the first hour after an oral glucose load (9). In this study, the first phase insulin response (measured as $\Delta ISR/\Delta G$ and $\Delta I/\Delta G$ during the first 30 min of OGTT, **Table 2**) was decreased only in O-IGT but not in O-NGT, compared to Y-NGT subjects. Similarly, O-IGT subjects had a reduction in the 0-120 min β cell response.

We investigated if the impairment in insulin release seen in O-IGT subjects is due to reduced incretin secretion. Against our prediction, we found that the incretin response was

upregulated in the older subjects (both O-NGT and O-IGT) compared to young. The higher GLP-1 response observed in both older groups might be interpreted as a physiological response to prevent loss of early phase insulin secretion. Nonetheless, this exaggerated GLP-1 response was not sufficient to normalize first phase insulin secretion in the O-IGT. Notably, β-cell sensitivity to glucose was altered only in older subjects with IGT, but not in those with NGT. This suggests resistance of the β-cells to the incretin effect such that the gut responds to the glucose load with an exaggerated GLP-1 release in an attempt to exert normal insulin secretion responses. A negative feedback relationship between insulin and GIP has been proposed to exist (28), but was never convincingly demonstrated, and the influence of insulin secretion on GLP-1 secretion is unclear.

A deleterious effect of aging *per se* on β cell function has not been consistently observed. For example, prior studies using the hyperglycemic clamp technique have shown little or no decrease in insulin secretion, both first and second phase, with aging (29-31). Our results are therefore in line with previous findings showing that, when early insulin secretion is preserved, older subjects have normal glucose tolerance.

The exaggerated GLP-1 response shown in this study is in line with the study from Ranganath et al (32). Yet, others did not find any difference in GLP-1 concentration between younger healthy controls vs. older healthy controls and older subjects with T2DM (33). Unlike our study, these previous studies did not account for the level of insulin resistance (34) and did not exclude subjects with positive family history for type 2 diabetes that are known to affect incretin responses (35). We found that the incretin response to the glucose load is enhanced in older subjects (NGT and IGT) compared with young controls, but β cell sensitivity to glucose is altered only in older subjects with IGT. This indicates a resistance of the β-cell to the incretin effect and therefore the gut may respond to the glucose load with an increased incretin release to obtain similar insulin responses, although the mechanism for this remains obscure. We propose that with aging the β cell becomes resistant to the incretin effect, thus requiring an increased release of GLP-1 and GIP to stimulate adequate insulin secretion in response to the glucose load.

Conclusions

We conclude that the incretin response in older subjects is not impaired, but rather increased. The insulin response in older NGT group is similar to the young NGT group, suggesting that resistance of the β cell to the incretin effect could be a mechanism contributing to the glucose intolerance seen with aging.

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Author contributions,

JG and RL performed the studies

JG, AG, JH, and NM analyzed the data and wrote the manuscript,

JH and RAD contributed to revising and reviewing the manuscript.

NM is the guarantor of this work and takes full responsibility for the integrity of information and concepts presented in the manuscript.

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Disclosure

José de Jesús Garduno-Garcia is a Speaker of Novo-Nordisk, Sanofi Aventis, Astrazeneca, Boheringer ingelheim, and Janssen; Amalia Gastaldelli is consultant for Eli-Lilly, Menarini, Gilead, Inventiva and Sanofi; Raweewan Lertwattanarak declares no conflict of interest for this article; Ralph A. DeFronzo is a member of the Advisory Board of Takeda, Bristol Myers Squibb, Janssen, Boehringer Ingelheim, Novo Nordisk, and Amylin. RAD is a member of the Speaker Bureau of Novo Nordisk, Amylin, BMS, and Janssen, RAD has grant support from Takeda, Amylin, and BMS. Dr. Musi and DeFronzo's salary are paid, in part, by the South Texas Veteran Healthcare System. Jens Juul Holst is member of advisory boards for MSD and NovoNordisk.

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Figure 1: OGTT glucose and insulin concentrations, and insulin secretion rates in Y-NGT, O-NGT and O-IGT non obese subjects. * p<0.05 O-IGT vs Y-NGT, § p<0.05 O-IGT vs O-NGT

Figure 2: Top panels: OGTT GLP-1 and GIP concentrations in Y-NGT, O-NGT and O-IGT non obese subjects. * p<0.05 O-IGT vs Y-NGT, § p<0.05 O-NGT vs Y-NGT, p=ns O-IGT vs O-NGT. Bottom panels: comparison of incremental ISR between 0-60min vs incremental GLP-1 and GIP concentrations. # p<0.05 changes in dAUC-ISR vs Y-NGT, ¶ p<0.05 changes in dAUC-GLP-1 or GIP vs Y-NGT. * p<0.05 changes in dAUC-ISR O-NGT vs O-IGT, § p<0.05 changes in dAUC-GLP-1 or GIP O-NGT vs O-IGT.

Table 1. Baseline Subjects' Characteristics

	Y-NGT	O-NGT	O-IGT	P§
Number	40	32	21	
Age (years)	25.4±3.4	71.9±7.2 #	76.6±6.7#	<0.0001
Sex (F/M)	26/14	14/19	11/9	NS
BMI (kg/m$^{2)}$	23.8±2.5	25.2±2.9	23.8±2.8	0.16
HbA1C (%)	5.1±0.3	5.5± 0.3v	5.6±0.3#	<0.0001
Insulin (pmol/l)	36.8±4.9	35.9±3.5	44.3±7.8	0.46
Glucose (mmol/L)	5.06± 0.55	5.36±0.54#	5.52±0.46#	0.001
Total Cholesterol (mmol/L)	3.89±0.17	4.39±0.23	4.40±0.17#	0.04
HDL Cholesterol (mmol/L)	1.43±0.08	1.51±0.9	1.54±0.11	0.39
LDL Cholesterol (mmol/L)	1.98± 0.18	2.43±0.20	2.41±0.13#	0.07
Triglycerides (mmol/L)	1.07±0.17	0.97±0.18	1.05±0.08*	0.91

# p<0.05 vs Y-NGT, * p<0.05 vs O-NGT, § old vs young

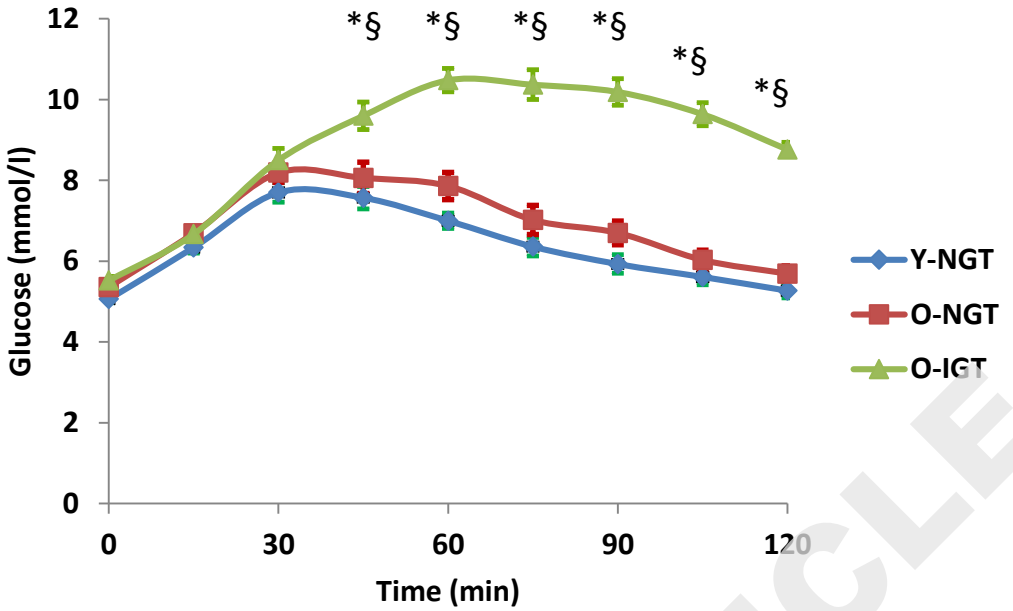
Table 2. OGTT indexes of insulin sensitivity and β cell function

	Y-NGT	O-NGT	O-IGT	P §
Glucose excursions during OGTT				
$\Delta G_{0\text{-}30}$ (mmol/l)	39.3±3.0	41.2±3.1	39.6±4.0	0.74
$\Delta G_{30\text{-}120}$ (mmol/l)	129.5±17.2	156.8±19.0	387.0±21.4#*	0.0001
Insulin sensitivity				
HOMA-IR	1.21±0.18	1.26±0.13	1.56±0.27	0.17
Matsuda index	11.0±1.4	8.1±0.7	6.5±0.7#	0.009

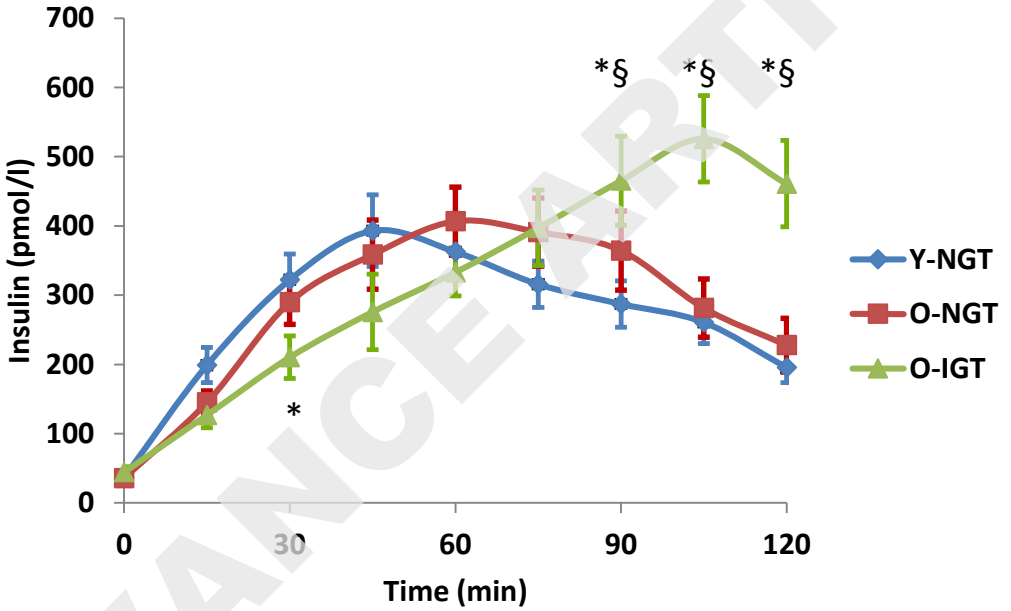
Insulin concentrations and secretion during OGTT				
ΔI_{0-30} (nmol/l)	4.6±0.6	3.6±0.5	2.5±0.5 #	0.03
ΔI_{30-120} (nmol/l)	24.9±2.6	27.7±3.6	31.0±4.5#	0.48
ΔISR_{0-30} (nmol/min)	22.6±2.2	22.6±2.4	13.7±2.2#*	0.02
ΔISR_{30-120}(nmol/min)	83.8±5.2	101.6±7.8	111.8±8.4#	0.02
$(\Delta I/\Delta G)_{0-30}$ (pmol/mmol)	127±20	102±17	68±16 #	0.02
$(\Delta I/\Delta G)_{30-120}$ (pmol/mmol)	578±479	415±119	84±12 #*	0.002
$(\Delta ISR/\Delta G)_{0-30}$ (nmol/min / mmol/l)	0.710±0.099	0.683±0.140	0.380±0.073 #*	0.004
$(\Delta ISR/\Delta G)_{30-120}$ (nmol/min / mmol/l)	4.327±4.429	1.480±0.403	0.300±0.025 #*	<0.0001
β cell function				
Rate sensitivity (Kd) pmol $\cdot m^{-2} \cdot mM^{-1}$	4097±1011	3560±1379	1919±508	0.03
Glucose sensitivity, pmol $\cdot min^{-1} \cdot m^{-2} \cdot mM^{-1}$	509±55	479±65	266±28#*	0.04
$(\Delta I/\Delta G)_{0-30}$ x Matsuda	1134±176	752±141	358±74 #*	<0.0005
$(\Delta I/\Delta G)_{0-120}$ x Matsuda	4026±1864	1685±351	462±63 #*	<0.001
$(\Delta ISR/\Delta G)_{0-30}$ x Matsuda	7.9±1.7	5.1±1.1	2.3±0.4 #*	<0.0002
$(\Delta ISR/\Delta G)_{0-120}$ x Matsuda	17.9±5.9	7.4±1.7	1.9±0.3 #*	<0.0001

# p<0.05 vs Y-NGT, * p<0.05 vs O-NGT, § old vs young

A



B



C