Determinants of Insulin Secretion After Renal Transplantation

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The high prevalence of post-transplant glucose intolerance and insulin resistance (IR) is associated with older age, family history of diabetes, immunosuppressive drugs, and antihypertensive therapy. However, the potential determinants of post-transplant β-cell dysfunction are largely unknown. The objective of the present study was to address this issue in detail. A total of 167 previously nondiabetic renal transplant recipients underwent a 75-g oral glucose tolerance test (OGTT)10 weeks after transplantation. Serum glucose and insulin were measured at 0, 1, and 2 hours. Three insulin release indices ($Secr_{AUC}$, $Secr_{1.phase}$, and $Secr_{2.phase}$) were calculated to assess the insulin secretory response as the dependent variable. To account for variations in insulin sensitivity (IS), β-cell function was also estimated as the disposition index (DI); the product of the IS index (ISI_{TX}) and $Secr_{1.phase}$. Increasing age was strongly and independently associated with a blunted insulin secretory response even after adjustment for IS ($P = .001$). An 80-year-old recipient had an approximately 50% lower insulin release than a 20-year-old individual, based on the linear regression model. Cytomegalovirus (CMV) disease and treatment with furosemide were both independently associated with β-cell dysfunction (DI; $P < .001$ and $P = .008$). Patients treated with angiotensin-converting enzyme (ACE)-inhibitors had an enhanced absolute insulin release, but the DI was similar in both treated and untreated recipients. We conclude that older age is an important determinant of β-cell dysfunction after renal transplantation. CMV disease and treatment with furosemide may also negatively influence pancreatic insulin release in renal transplant recipients.

THE HIGH PREVALENCE of post-transplant glucose intolerance has been reported to be associated with age, family history of diabetes, treatment with corticosteroids and calcineurin inhibitors, and antihypertensive therapy.[1-3] Moreover, cytomegalovirus (CMV) disease and hepatitis C may increase the risk for the development of post-transplant diabetes mellitus (PTDM).[2,4]

In the general population it has been established that genetic factors influence both insulin secretion[5] and insulin action,[6,7] and that increasing age may be associated with impaired insulin release.[8,9] Furthermore, obesity and the associated elevated levels of triglycerides and free fatty acids have been reported to adversely affect both insulin sensitivity (IS)[10] and insulin secretion.[6,11,12] Treatment with β-blockers increases the risk for type 2 diabetes[13] and both impaired insulin release and insulin resistance (IR) are suggested mechanisms for this.[14] Thiazide diuretics worsen IR, but several reports have also described impaired insulin release as an untoward effect of these drugs.[14-16] Angiotensin-converting enzyme (ACE)-inhibitors may have beneficial effects on insulin release and insulin action,[14-18] whereas sustained-release calcium channel blockers probably are neutral.[14]

Post-transplant glucose intolerance is caused by impaired IS accompanied with an insufficient insulin secretory response.[19,20] Immunosuppressive and antihypertensive drugs may influence both insulin release and insulin action in this condition. Recently, we reported that body mass index (BMI), daily prednisolone dose, CMV disease, and triglycerides were independent predictors of post-transplant IR, whereas the use of β-blockers and diuretics also had a negative effect on IS after renal transplantation.[3]

It has been argued that both the calcineurin inhibitors cyclosporine A (CsA) and tacrolimus reduce insulin secretion.[1,21-23] The inhibitory effect of tacrolimus on the pancreatic β cell is probably dose-dependent,[1,21-23] whereas it remains unclear whether the diabetogenic effect of CsA depends on dose or whole-blood trough levels.

Although candidate risk factors such as age, genetic predisposition, CMV disease, immunosuppressive therapy, and antihypertensive medication may negatively influence β-cell function after renal transplantation, no previous study has addressed this issue in detail.

The primary objective of the present study was to test the hypothesis that risk factors such as age, a family history of diabetes, CMV disease, and higher concentrations of whole-blood CsA are associated with impaired insulin secretion in renal transplant recipients. The secondary aim was to assess the influence of different antihypertensive drugs on insulin secretion.

MATERIALS AND METHODS

The study population, analytical procedures, and immunosuppressive protocol have been described in detail.[3] Briefly, a total of 167 consecutive previously nondiabetic patients (117 men) underwent a 75-g oral glucose tolerance test (OGTT) 10 weeks (70 ± 6 days) after renal transplantation. Sixty-six patients (40%) received a kidney from a living donor. All patients gave informed consent to participate, and the study was performed in accordance with the Declaration of Helsinki.[24]

All patients received prednisolone, 162 patients received CsA (Sandimmun Neoral, Sandoz Pharma, Basel, Switzerland), and 4 tacrolimus. Eighty-three patients (50%) had normal glucose tolerance (NGT), 5 (3%) had impaired fasting glucose (IFG), 50 (30%) had impaired glucose tolerance (IGT), and 29 (17%) had PTDM.

Patient characteristics according to the category of glucose tolerance are given in Table 1. The patients had a mean age of 47 years (SD = 16; range, 18 to 80) and a BMI of 23.5 kg/m^2 (SD = 3.8; range, 12.8

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Table 1. Patient Characteristics in the Three Groups With Varying Degrees of Glucose Tolerance

	PTDM	IGT/IFG	NGT	*P*
No. of patients	29 (17%)	55 (33%)	83 (50%)	
Age (yr)	54 ± 15	49 ± 16	43 ± 15	.003
BMI (kg/m^2)	24.4 ± 4.0	23.6 ± 3.9	23.0 ± 3.6	.210
Serum triglyceride level (mmol/L)	2.6 ± 1.4	2.3 ± 1.0	1.8 ± 0.8	<.001
Prednisolone dose (mg/d)	16.9 ± 7.1	17.1 ± 7.3	13.3 ± 5.4	.001
CsA concentration (μg/L)	242 ± 46	255 ± 81	234 ± 47	.167
No. of patients with:				
Family history of diabetes	8 (28%)	11 (21%)	12 (15%)	.326
HLA B27 phenotype	8 (28%)	8 (15%)	9 (11%)	.093
CMV disease	10 (35%)	18 (33%)	14 (17%)	.049
No. of patients treated with:				
Furosemide	13 (45%)	26 (48%)	20 (24%)	.008
β-blockers	17 (59%)	29 (54%)	23 (28%)	.001
ACE-inhibitors	5 (17%)	19 (35%)	23 (28%)	.221

NOTE. Values are presented as mean ± SD or numbers of patients (percentage). Statistics: for continuous data 1-way ANOVA; for categorical data, Pearson chi-square.

Abbreviations: BMI, body mass index; CsA, cyclosporine A; CMV, cytomegalovirus; ACE, angiotensin-converting enzyme; PTDM, post-transplant diabetes mellitus; IGT, impaired glucose tolerance; IFG, impaired fasting glucose; NGT, normal glucose tolerance.

to 39.2). The mean daily prednisolone dose was 15 mg (SD = 7; range, 10 to 30), daily CsA dose 342 mg (SD = 115; range, 175 to 1,000), whole blood CsA trough level 242 μg/L (SD = 60; range, 110 to 535), and serum creatinine 145 μmol/L (SD = 44; range, 64 to 350). Fifty-seven percent of the recipients had been treated for one or more rejections and 25% for CMV disease. Nineteen percent (n = 31) of the patients had a first-degree relative with diabetes mellitus. One hundred forty patients (84%) were hypertensive (hypertension defined as repeated blood pressure measurements > 140/90 or the use of antihypertensive medication), of whom 130 were treated with antihypertensive drugs. Sixty-nine patients (41%) received a β-blocker, 59 (35%) furosemide, 68 (41%) a calcium-channel antagonist, 47 (28%) an ACE-inhibitor, and 23 patients (14%) were treated with an α blocker.

After an overnight fast serum glucose and insulin were measured at 0, 1, and 2 hours during a 75-g OGTT. Serum glucose was analyzed using a glucose dehydrogenase method (Cobas Mira, Roche, Basel, Switzerland), whereas serum insulin was determined by a commercial radioimmunoassay (Coat-A-Count, Diagnostic Products Corp, Los Angeles, CA). Whole-blood CsA trough concentrations were measured using a CsA-specific fluorescence polarization immunoassay (TDx analyzer, Abbott Laboratories, Chicago, IL).

Insulin Release and Insulin Sensitivity Indices

Insulin release was estimated by the use of three equations documented to correlate well (r = 0.70 to 0.75) with insulin secretion as assessed by hyperglycemic clamp studies in patients with varying degrees of glucose tolerance.[25-27] The area under curve (AUC) insulin and the AUC glucose during the OGTT, were calculated using the trapezoid rule, and implemented in the insulin release index; $Secr_{AUC} = AUC_{Ins}/AUC_{Gluc}$.[25-27] The first-phase and second-phase insulin releases were estimated implementing insulin values at 0 and 60 minutes, and glucose at 60 minutes: $Secr_{1.phase} = 1{,}194 + 4.724 \cdot Ins_0 - 117.0 \cdot Gluc_{60} + 1.414 \cdot Ins_{60}$ and $Secr_{2.phase} = 295 + 0.349 \cdot Ins_{60} - 25.72 \cdot Gluc_{60} + 1.107 \cdot Ins_0$.[26]

We have recently shown that the OGTT-derived IS index ISI_{TX} ($= 0.208 - 0.0032 \cdot BMI - 0.0000645 \cdot Ins_{120} - 0.00375 \cdot Gluc_{120}$) correlates closely with the results from euglycemic hyperinsulinemic glucose clamp studies (r = 0.58, $P < .001$).[3]

The acute insulin secretory response increases with decreasing insulin action to maintain NGT, and the relationship between insulin release and IS has been described as hyperbolic.[6,28-30] The product of the estimates of IS and insulin release, known as the disposition index (DI), is a constant in normoglycemic individuals, whereas the development of glucose intolerance is associated with a decline of the DI.[6] In other words, the DI describes the ability of the pancreatic β cell to compensate for various degrees of IR, and may therefore represent a more appropriate measure of β-cell function than the absolute insulin release. In the present study the DI was estimated as the product of the first-phase insulin release and the ISI_{TX}.

Statistical Analysis

Data are given as medians and range or means and standard deviations. Mann-Whitney test, 1-way analysis of variance (ANOVA), or unpaired t test was used to compare groups as appropriate. Skewed variables were ln-transformed before analysis with parametric methods. Linear regression was implemented in the analyses of potential independent continuous variables compared with the ln-transformed insulin release indices as the dependent variables. Multiple linear regression was used to assess the potential determinants for insulin secretion as the dependent variable. All variables associated with any insulin release index in the univariate analysis with a P value < .1 were included in a forward stepwise multiple regression model. All statistical tests of significance were 2-tailed and P values < .05 considered significant. Particular attention should however be directed towards P values < .01 since a considerable number of P values have been calculated. The analysis was implemented using SPSS version 10.0 (SPSS Inc, Chicago, IL).

RESULTS

Increasing age was uniformly and significantly associated with a blunted insulin secretory response ($Secr_{AUC}$: B = −0.010, r^2 = 0.09, and $P < .001$; $Secr_{1.phase}$: B = −0.012, r^2 = 0.07, and $P < .001$; $Secr_{2.phase}$: B = −0.010, r^2 = 0.09, and $P < .001$) (Fig 1A through C), whereas higher levels of serum triglycerides were associated with a decrease in $Secr_{AUC}$ (B = −0.080, r^2 = 0.02, and P = .049). No significant associations were found between CsA trough concentration, daily prednisolone dose, BMI, and insulin secretion.

Patients who received an ACE-inhibitor had a higher median insulin release than those who did not: $Secr_{AUC}$ (P = .007),

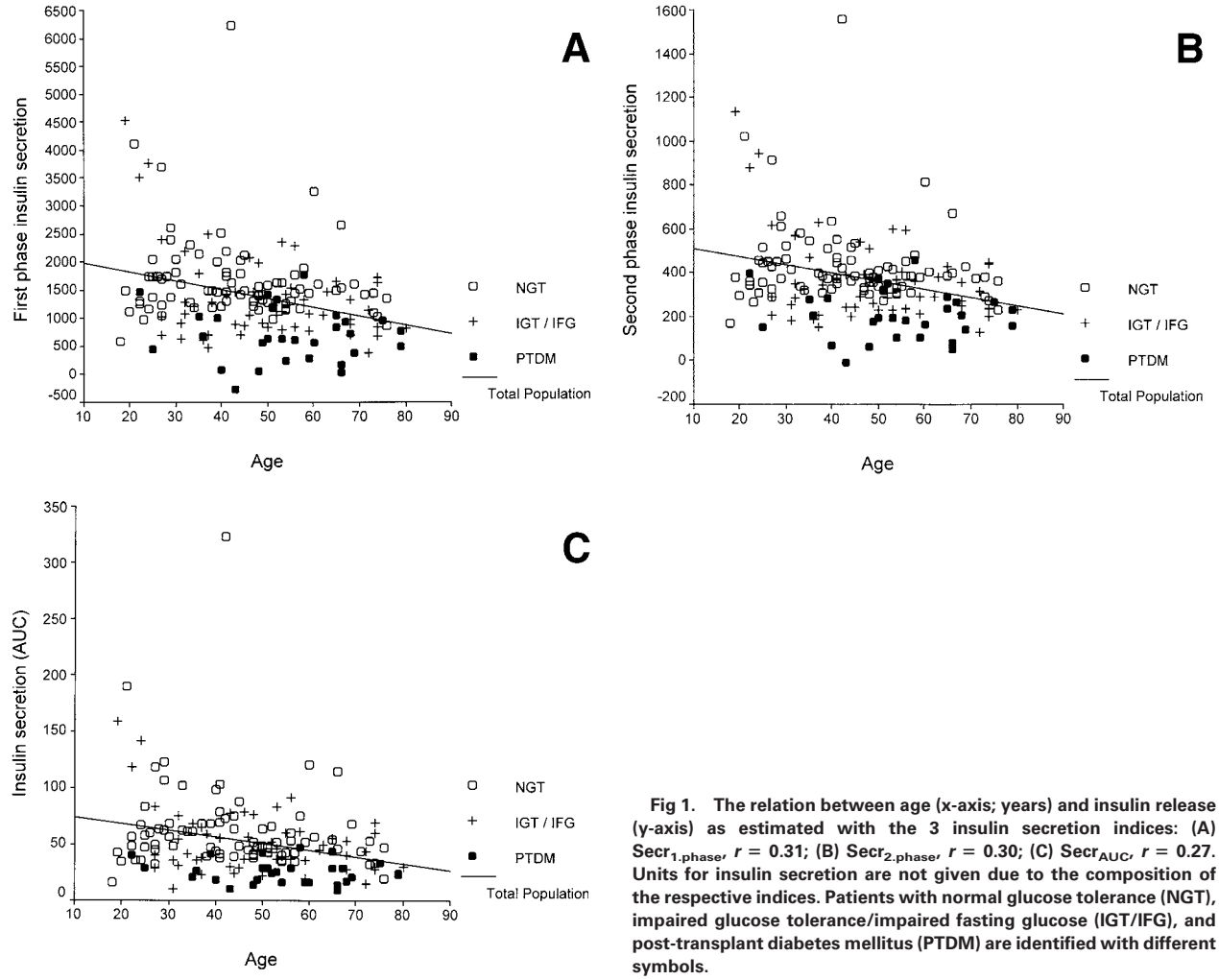

Fig 1. The relation between age (x-axis; years) and insulin release (y-axis) as estimated with the 3 insulin secretion indices: (A) $Secr_{1.phase}$, $r = 0.31$; (B) $Secr_{2.phase}$, $r = 0.30$; (C) $Secr_{AUC}$, $r = 0.27$. Units for insulin secretion are not given due to the composition of the respective indices. Patients with normal glucose tolerance (NGT), impaired glucose tolerance/impaired fasting glucose (IGT/IFG), and post-transplant diabetes mellitus (PTDM) are identified with different symbols.

$Secr_{1.phase}$ ($P = .015$), and $Secr_{2.phase}$ ($P = .013$) (Table 2). There was a trend towards lower insulin release in the group of patients treated with furosemide ($Secr_{1.phase}$: $P = .104$; $Secr_{2.phase}$: $P = .121$).

The patients who had suffered from CMV disease had a lower $Secr_{1.phase}$ and $Secr_{2.phase}$ ($P = .039$ and 0.047, respectively) than the others, and the group with a positive HLA B27 phenotype had a lower median $Secr_{AUC}$ ($P = .022$) than patients without this specific phenotype (Table 2).

A family history of diabetes, whole-blood CsA trough concentration, smoking status, gender, serum creatinine, and treatment with β-blockers were not significantly associated with changes in insulin secretion in the univariate model.

After forward stepwise multiple linear regression analysis,

Table 2. Median Insulin Secretion in Different Groups of Patients

	$Secr_{AUC}$			$Secr_{1.phase}$			$Secr_{2.phase}$		
	Yes	No	*P*	Yes	No	*P*	Yes	No	*P*
Patients with:									
Family history of diabetes	42	45	.259	1,296	1,303	.408	339	348	.401
HLA-B27 phenotype	36	46	.022	1,023	1,314	.154	278	351	.155
CMV disease	41	45	.245	1,127	1,390	.039	304	359	.047
Patients treated with:									
Furosemide	40	47	.164	1,111	1,415	.104	296	367	.121
β-blockers	43	47	.643	1,209	1,364	.572	324	357	.572
ACE-inhibitors	52	42	.007	1,428	1,219	.015	374	323	.013

NOTE. Estimated median insulin secretion is given in different groups of patients categorized as "yes" or "no" describing the values of the respective categorical variables in the left column. Statistics: Mann-Whitney test.

age remained strongly and significantly associated with impaired insulin release after the implementation of all 3 insulin release indexes ($Secr_{AUC}$: $P < .001$; $Secr_{1.phase}$: $P = .001$; and $Secr_{2.phase}$: $P < .001$). Insulin secretion, as assessed by the 3 insulin secretion indices in the linear regression model, declined 45% to 50% from the age of 20 to 80. The serum concentration of triglycerides was inversely associated with $Secr_{AUC}$ ($P = .033$) also after multivariate analysis. ACE-inhibitor therapy remained associated with significantly higher $Secr_{2.phase}$ ($P = .032$), whereas the association with higher $Secr_{1.phase}$ ($P = .067$) and $Secr_{AUC}$ ($P = .104$) was weakened after multivariate analysis. The negative association between CMV disease and insulin release failed to reach statistical significance in the multiple linear regression model ($Secr_{1.phase}$: $P = .149$; $Secr_{2.phase}$: $P = .088$).

β-Cell function assessed as the DI ($Secr_{1.phase} \cdot ISI_{TX}$), was inversely associated with increasing age (B = −0.90, $P = .001$, $r^2 = 0.07$), daily prednisolone dose (B = −1.47, $P = .023$, $r^2 = 0.03$), BMI (B = −4.29, $P < .001$, $r^2 = 0.09$), and triglycerides (B = −9.42, $P = .021$, $r^2 = 0.03$). The patients who received furosemide had a lower mean DI than those who did not (unpaired *t* test; 79 *v* 113, $P < .001$), and patients who had been treated for CMV disease also had a lower DI than others (79 *v*108, $P = .003$). The HLA B27 phenotype group of patients had a significantly lower mean DI (78 *v* 104, $P = .026$).

After multiple linear regression analysis, increasing age (B = −0.83, $P = .001$), CMV disease (B = 31.8, $P < .001$), BMI (B = −4.42, $P < .001$), and diuretic therapy (B = 21.6, $P = .008$) remained significantly associated with a diminished DI.

DISCUSSION

Older Age Impairs Insulin Release in Renal Transplant Recipients

Our results indicate that increasing age is a major determinant of impaired insulin release after renal transplantation. The association between older age and β-cell dysfunction was consistent and independent of other potential confounding variables including variations in IS. This finding is also in accordance with the results of recently published studies including healthy individuals.[8,9] We report an approximately 50% lower insulin release in a patient aged 80 as compared with a 20-year-old recipient. This is comparable with the 25% decline in insulin delivery over the range from 18 to 85 years reported in the former study,[8] and the 1% decline in insulin release per year reported in the latter study.[9]

The mechanism of impaired insulin release in older individuals is not clearly understood. However, the aging of β cells has been characterized by a reduction in mass and disturbances in different insulin secretory patterns.[9] Recently, it has been argued that the presence of islet cell autoimmunity (GAD65 and IA-2 autoantibodies) may be associated with impaired insulin secretion in older type 2 diabetic subjects.[31] Moreover, the age effect on insulin secretion has also been suggested to be attributable to impaired proinsulin conversion to insulin.[32]

Age is known to be an independent predictor of post-transplant glucose intolerance,[1,2] but is not associated with IR after adjustment for BMI.[3,9,32] Given the results of the present study, we suggest that β-cell dysfunction is a major pathogenetic mechanism leading to glucose intolerance with increasing age in renal transplant recipients.

CMV Disease

The present data indicate that CMV disease is associated with β-cell dysfunction. First, patients who had been treated for CMV disease had a lower median insulin release than the others as seen in the univariate analyses. Second, the DI was significantly lower in the patients who had been treated for CMV disease also after multiple linear regression ($P < .001$), suggesting that these patients had an inappropriate insulin secretory response relative to the degree of IR.

We have previously shown that CMV disease predicts IR[3] and PTDM[2] in renal transplant recipients. Consequently, when taking the present results into account, it can be argued that CMV disease may cause PTDM through both impaired β-cell function and insulin action. The hypothesis that virus infections may cause diabetes type 1 is well known, but there have been few reports on CMV infection specifically. In 1988, Pak et al reported a strong correlation between the CMV genome and islet cell autoantibodies in newly diagnosed type 1 diabetic patients.[33] Moreover, it has been argued that human CMV may play an important role in the pathogenesis of type 1 diabetes through T-cell cross-reactivity with GAD65.[34] CMV can also infect pancreatic islet cells in vivo.[34]

The high prevalence of ganciclovir-treated CMV disease in our transplant population may have unraveled potential pathogenetic mechanisms for diabetes that have been difficult to discover in the general population.

Diuretics and ACE-Inhibitors

The patients who received furosemide had a significantly lower insulin response than those who did not when adjusted for their tendency towards lower IS. This is in accordance with findings in hypertensive patients in the general population. The use of thiazide-diuretics has been associated with both reduced IS and impaired β-cell function,[14-16] and potassium depletion may be one pathogenetic mechanism.[15,16] Moreover, in a double-blind crossover study of 23 hypertensive patients, furosemide 60 mg daily diminished the early insulin response to intravenous glucose.[35]

ACE-inhibitor–treated patients showed a tendency towards enhanced absolute insulin release also after multivariate analysis, but when the effect on IS was taken into account no significant difference was found between treated and untreated patients. Previous studies have indicated that ACE-inhibitors actually may increase the insulin secretory response as assessed by the intravenous[15] or oral[17] glucose tolerance test.

The mechanism for the potential beneficial effects of ACE-inhibitors on early insulin release is not fully understood. One plausible hypothesis is that ACE-inhibition induces increased islet blood flow, which is important to secure a sufficient early insulin response.[18] Another is that ACE-inhibitor therapy is associated with higher potassium levels, which may augment the insulin secretory response.[16,17]

Body Mass Index

Increasing BMI was associated with pancreatic β-cell dysfunction (DI). This is in accordance with findings in the general population.[6,36] The potential negative effects of obesity on β-cell function may be mediated through the increase in free fatty acids and triglycerides, commonly observed in obese individuals.[6,11] Nonesterified free fatty acids may be detrimental by inducing apoptosis in the β cell.[11] In the present study higher levels of serum triglycerides were significantly associated with impaired insulin release ($Secr_{AUC}$) also after adjustment for changes in IS in the univariate model.

Family History of Diabetes

We did not find any significant association between a family history of diabetes and β-cell dysfunction even though this has been documented in the general population.[5,7] However, the present study was not designed to have sufficient power to detect a genetic disposition for impaired insulin release.

Limitations

The present study has some limitations which should be taken into account when interpreting the results. First, even though our main findings were in accordance with the present knowledge from the general population, the cross sectional design makes it difficult to establish any causal relationships. A longitudinal study should be performed to test our hypotheses.

Second, compared to insulin measures derived from hyperglycemic clamp studies or intravenous glucose tolerance tests, the surrogate estimates for insulin release implemented in the present study may be inferior. However, the insulin release indices have been extensively validated in patients with different degrees of glucose tolerance from normal to diabetes,[25-27] and are more suitable and less expensive, when evaluating a large number of patients.

Furthermore, it has been argued that the OGTT derived IS and insulin release indices do not perfectly adjust to a hyperbolic relation.[37] However, van Haeften and Stumvoll reported that even though the estimated first-phase insulin release ($Secr_{1.phase}$) appears to reach a maximum in insulin resistant subjects, the second-phase insulin release ($Secr_{2.phase}$) yielded a perfect hyperbolic relation with the IS index.[38] To further elaborate this issue, we implemented a second DI based on the second-phase insulin secretion ($DI_2 = Secr_{2.phase} \cdot ISI_{TX}$) in the statistical calculations. The results were in fact almost identical with the results from the DI derived from the first-phase insulin implemented in the present study.

In addition, we performed calculations that documented a hyperbolic relation between ISI_{TX} and $Secr_{1.phase}$ in the renal transplant recipients with NGT (data not shown).

Finally, although we found a highly significant and independent association between increasing age and decreasing insulin secretion, the relatively low r^2 values (0.07 to 0.09) indicate that other factors explain most of the variations in insulin secretion. Differences in age alone explained 7% to 9% of the variation in insulin release.

Conclusions

Older age is an important determinant of impaired β-cell function after renal transplantation. CMV disease, diuretic therapy, and obesity may also negatively influence β-cell function, whereas treatment with ACE-inhibitors seems to enhance posttransplant insulin release.

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