# Islet Transplantation Stabilizes Hemostatic Abnormalities and Cerebral Metabolism in Individuals With Type 1 Diabetes

*Francesca D'Addio,[1,2] Paola Maffi,[2] Paolo Vezzulli,[3] Andrea Vergani,[1] Alessandra Mello,[4] Roberto Bassi,[1] Rita Nano,[5] Monica Falautano,[6] Elisabetta Coppi,[6] Giovanna Finzi,[7] Armando D'Angelo,[8] Isabella Fermo,[8] Fabio Pellegatta,[9] Stefano La Rosa,[7] Giuseppe Magnani,[6] Lorenzo Piemonti,[5] Andrea Falini,[3] Franco Folli,[10] Antonio Secchi,[2,11] and Paolo Fiorina[1,2]*

## OBJECTIVE

**Islets after kidney transplantation have been shown to positively affect the quality of life of individuals with type 1 diabetes (T1D) by reducing the burden of diabetes complications, but fewer data are available for islet transplantation alone (ITA). The aim of this study was to assess whether ITA has a positive impact on hemostatic and cerebral abnormalities in individuals with T1D.**

## RESEARCH DESIGN AND METHODS

**Prothrombotic factors, platelet function/ultrastructure, and cerebral morphology, metabolism, and function have been investigated over a 15-month follow-up period using ELISA/electron microscopy and magnetic resonance imaging, nuclear magnetic resonance spectroscopy, and neuropsychological evaluation (Profile of Mood States test and paced auditory serial addition test) in 22 individuals with T1D who underwent ITA (*n* = 12) or remained on the waiting list (*n* = 10). Patients were homogeneous with regard to metabolic criteria, hemostatic parameters, and cerebral morphology/metabolism/function at the time of enrollment on the waiting list.**

## RESULTS

**At the 15-month follow-up, the group undergoing ITA, but not individuals with T1D who remained on the waiting list, showed *1*) improved glucose metabolism; *2*) near-normal platelet activation and prothrombotic factor levels; *3*) near-normal cerebral metabolism and function; and *4*) a near-normal neuropsychological test.**

## CONCLUSIONS

**ITA, despite immunosuppressive therapy, is associated with a near-normalization of hemostatic and cerebral abnormalities.**

*Diabetes Care 2014;37:267–276 | DOI: 10.2337/dc13-1663*

Type 1 diabetes (T1D) is associated with microvascular (e.g., retinopathy, nephropathy, and neuropathy) or macrovascular (e.g., cardiovascular, cerebrovascular, and peripheral vascular disease) complications, which cause an increase in morbidity and mortality and negatively affect patient quality of life (1,2). Poor glycemic control is the primary contributor to the onset of chronic complications (3).

[1]*Nephrology Division, Boston Children's Hospital, Harvard Medical School, Boston, MA*
[2]*Department of Transplant Medicine, San Raffaele Hospital, Milan, Italy*
[3]*Department of Neuroradiology, San Raffaele Hospital, Milan, Italy*
[4]*Department of Anesthesiology, San Raffaele Hospital, Milan, Italy*
[5]*Diabetes Research Institute, San Raffaele Hospital, Milan, Italy*
[6]*Department of Neurology, San Raffaele Hospital, Milan, Italy*
[7]*Pathology Department, Ospedale di Circolo, Varese, Italy*
[8]*Chromatographic Techniques Service, San Raffaele Hospital, Milan, Italy*
[9]*Centre for the Study of Atherosclerosis, Bassini Hospital, Cinisello Balsamo, Italy*
[10]*Department of Medicine, Division of Diabetes, University of Texas at San Antonio, San Antonio, TX*
[11]*Department of Medicine, Universita' Vita Salute San Raffaele, Milan, Italy*

*Corresponding author: Paolo Fiorina, paolo.fiorina@childrens.harvard.edu, or Antonio Secchi, antonio.secchi@hsr.it.*

*Received 15 July 2013 and accepted 30 August 2013.*

*This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc13-1663/-/DC1.*

*© 2014 by the American Diabetes Association. See http://creativecommons.org/licenses/by-nc-nd/3.0/ for details.*

By replenishing destroyed pancreatic islets, islet transplantation leads to improved glycometabolic control—if not to improved insulin independence—and reduces the frequency of hypoglycemic episodes, thus delaying diabetes complications and improving patient quality of life (4–6). However, islet transplantation requires the chronic administration of immunosuppressive agents (7), which increase cardiovascular disease (8), atherosclerosis (9), and dyslipidemia (10) and are toxic to β-cells, thus shortening the life span of the transplanted islets (11,12). In the islet after kidney transplantation group, patients who received a kidney transplantation received immunosuppressive therapy before transplantation of the islet graft, whereas individuals with T1D receiving an islet allograft need to begin an immunosuppression regimen at the time of the transplant. It is therefore necessary to demonstrate that the benefit obtained from improved glycometabolic control overcomes the deleterious effect of immunosuppression. Indeed, islet transplantation after kidney transplantation demonstrated an overall beneficial effect in halting the progression of several major diabetes complications (13) such as nephropathy (14,15) and cardiovascular disease (16), yet fewer data are available on the effect of islet transplantation alone (ITA) on diabetes complications (17,18).

In this study we examined the effect of ITA on hemostatic and cerebrovascular abnormalities in individuals with T1D. Hemostatic abnormalities are evident in T1D (19) and may contribute to the onset of accelerated atherosclerosis and microangiopathy of different vascular districts, including the cerebral vasculature, thus leading to morphological, metabolic, and functional cerebral abnormalities (20). Features of hemostatic abnormalities include enhanced activation of the clotting system and severe platelet function, which lead to a prothrombotic state, with vascular dysfunction/early atherosclerosis observed in several organs, particularly in the cerebral district, in individuals with T1D (20). The resulting diabetic encephalopathy is characterized by a reduction in the volume of the cerebral cortex; a significant loss of neocortical neurons (21); a decrease in mnemonic and abstract reasoning, problem-solving abilities, and hand-eye coordination; and an increased risk of Alzheimer disease (22). We hypothesized that improvement of glycometabolic control (if not full restoration of normoglycemia) obtained with islet transplantation may reestablish the physiology of the hemostatic system, thus halting the progression of cerebral abnormalities. The aim of this study was to investigate the relatively short-term effects of islet transplantation on hemostatic profile and platelet function as well as cerebral morphology (brain volume), metabolism (neuronal/axonal metabolism), and function (cognitive and neuropsychological function).

## RESEARCH DESIGN AND METHODS

### Patients

This nonrandomized pilot study included 22 individuals with T1D who were actively enrolled on the islet transplantation waiting list. Twelve of these individuals underwent ITA, while 10 remained on the waiting list (T1D-WL) (Supplementary Fig. 1). Individuals with T1D were retrospectively enrolled for a 24-month period according to the major criteria applied to enter the islet transplantation program, as published elsewhere (4,7). The baseline and demographic characteristics of the two groups of individuals with T1D were comparable (Supplementary Table 1) and were reexamined at 15 months of follow-up (15.5 $\pm$ 2.5 months). Glycometabolic parameters were determined at enrollment and during the follow-up using a single determination; thus they are only partially representative of the individual's glycometabolic control. Ten healthy volunteers matched for age and sex (control group [CTRL]) were studied as well. Patients with a history of cerebrovascular disease (transient ischemic attack/stroke) and/or who were taking an oral anticoagulant agent were excluded. All subjects provided informed consent before enrollment. Studies not included in the routine clinical follow-up were approved as appropriate by an institutional review board. ITA is associated with different adverse events because of both the procedure and immunosuppressive treatments. On the basis of our experience, acute complications are represented by thrombosis of a peripheral branch of the portal vein (9%) and bleeding (36%), whereas chronic complications may include decreased renal function (15%), viral myocarditis (6%), and reactivation of cytomegalovirus infections (6%) (23).

### Immunosuppression and Concomitant Therapy

Individuals who underwent islet transplantation received five doses of intravenous daclizumab 1 mg/kg (Zenapax, Roche) for 2 weeks as induction therapy. As maintenance, tacrolimus (Prograf, Fujisawa; serum levels of 3–6 ng/mL) and sirolimus (Rapamune, Wyeth-Ayerst; ranging from 12–15 ng/mL for 90 days and 7–10 ng/mL thereafter) were used. Four individuals were switched to a different immunosuppressive regimen because of side effects: either tacrolimus plus mycophenolate mofetil (MMF; $n$ = 2), sirolimus plus MMF ($n$ = 1), or cyclosporine plus MMF ($n$ = 1). Four of 12 individuals in the ITA group and 4 of 10 individuals with T1D received ACE inhibitors. One patient in the ITA group received statins during follow-up. No drugs were administered to controls.

To understand whether immunosuppression represents a potential bias in our study and whether it affects coagulatory/inflammatory markers, we considered an additional 10 individuals with end-stage renal disease (ESRD) and 10 individuals with ESRD who subsequently received a kidney transplant; these patients were studied at 15 months of follow-up and treated with thymoglobulin as induction therapy and tacrolimus plus sirolimus (or MMF in few cases) as maintenance therapy. Individuals with ESRD were all receiving hemodialysis treatment at our hospital; patients received ACE inhibitors (7 of 10), β-blockade agents (3 of 10), calcium-phosphate binders (10 of 10), and/or 1-OH vitamin D (8 of 10) as concomitant therapy. Finally, with the same purpose of understanding whether immunosuppression affects

coagulatory/inflammatory markers in a pure T1D population, we considered five individuals with T1D receiving sirolimus monotherapy (0.1 mg/kg) for 37–197 days (target levels 8–10 ng/m) and atorvastatin 10 mg for 44–279 days as preconditioning for islet transplantation (at least 30 days).

### Laboratory Analysis

Platelet-poor plasma was obtained by centrifugation at room temperature (10 min at 1,500 g). Determinations of prothrombin time, activated partial thromboplastin time, fibrinogen (Fg), antithrombin, fasting homocysteine *d*-dimer fragments (D-dimer), levels of prothrombin fragments 1+2 (F1+2), protein C, and protein S were analyzed using fresh plasma samples (24).

### Intracellular Calcium in Platelets

Platelet-rich plasma was obtained by centrifugation of blood with 1 mL of anticoagulant citrate dextrose (ACD) solution and collection of supernatant. Platelet intracellular calcium ($[Ca^{2+}]i$) was evaluated under resting conditions as previously described (25).

### Electron Microscopy

Aliquots of platelet-rich plasma were fixed, and ultrastructural evaluation of four cases from each group included platelet size, morphology, and granule content, and platelet areas were measured using the Measure Arbitrary Area tool of Analysis Image Processing 3.0 software (Soft Imaging System, Münster, Germany).

### Magnetic Resonance Imaging and Spectroscopy Protocols

Magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) of protons ($^1$H-MRS) were performed using a 1.5-T scanner (Vision; Siemens, Erlangen, Germany) with a head-dedicated coil for scanning. Acquired images ($T_2$, $T_1$, axial fluid attenuated inversion recovery, and coronal spin) were analyzed to assess micro- and macroangiopathy during the clinical course of diabetes, as previously reported (26). A diagnosis of cerebrovascular disease was formulated when lesions were hyperintense in $T_2$-weighted and fluid attenuated inversion recovery images and hypointense on $T_1$-weighted images. $T_1$-weighted axial images of each participant were transferred to an offline workstation (Sun Sparcstation; Sun Microsystems, Mountain View, CA) for brain volume assessment (27). Single-voxel MRS was performed at least three times for each individual (28). The peaks of choline (Cho), creatine (Cr), and *N*-acetylaspartate (NAA) were calculated, and their integral function was solved by automatic and manual measurement. Results were expressed as ratios: NAA to Cr, Cho to Cr, and NAA to Cho. Cr was considered an internal standard because its levels are relatively uniform even in pathological conditions (29). All measurements were performed by the P.V.; whole-brain NAA MRS was analyzed as previously described (28).

### Psychological and Neuropsychological Tests

Psychological assessment evaluated emotional patterns and quality of life (QoL) of patients. The Profile of Mood State (POMS) was used to measure present mood states and disturbance symptoms, dissecting six dimensions of mood: tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, and confusion-bewilderment (30). To assess the patients' QoL, the LEIPAD questionnaire was administered, as reported by our group (28,31). A neuropsychologist performed a neuropsychological evaluation in one session by analyzing childhood intellectual failures, global cognitive efficiency, language, attention, reasoning, and verbal/visual-spatial memory, as previously reported (28). The Mini-Mental State Examination was used to examine patients' global (intellectual) cognitive abilities. Language functioning (oral comprehension and access to internal lexicon) was evaluated using the token test and phonemic and semantic word fluency test (32). Attention abilities were analyzed using the Stroop test (28) and the paced auditory serial addition test, which evaluated sustained attention and information processing speed (33). Verbal and visual short-term memory function was evaluated with the verbal span and Corsi span tests, whereas verbal long-term memory was assessed with short story recall. Finally, abstract reasoning was evaluated through visuospatial stimuli using the Wisconsin card sorting test (48-card form) (34). Evaluations were performed three times after extensive internal validation.

### Statistical Analysis

Continuous variables are presented as mean $\pm$ SD and compared with two-tail Student *t* test for unpaired data (two groups) or the one-way ANOVA (three or more groups); the Mann-Whitney *U* test (two groups) or the Kruskal-Wallis test (three or more groups) were used if no normal distribution was evident. Categorical variables are presented as proportions with 95% CIs and were compared using the $\chi^2$ test if five or more observations are in each cell, or the Fisher exact test if there are fewer than five observations. A $P$ value $< 0.05$ was considered statistically significant. CIs were estimated using the binomial exact. Analysis was performed using Stata software, version 11.1 (StataCorp, LP, College Station, TX).

## RESULTS

### Baseline Patient Characteristics

Individuals with T1D and healthy controls did not differ in any major demographic characteristics at baseline, when considering individuals with T1D who remained on the waiting list (T1D-WL) or those who underwent ITA (T1D-ITA) (Supplementary Table 1). The mean age, duration of diabetes, creatinine level, total cholesterol, HDL cholesterol, exogenous insulin requirement, and mean arterial pressure were similar in the three groups of subjects examined (Supplementary Table 1). Significant differences were observed in the major parameters accounting for glycometabolic control between individuals with T1D (T1D-WL and T1D-ITA) and controls, including glycated hemoglobin ($HbA_{1c}$), serum glucose (*S*-glucose) and C-peptide, whereas no differences were found when comparing the values of the T1D-WL versus the T1D-ITA groups (Supplementary Table 1). All individuals with T1D (T1D-WL and T1D-ITA) were homogenous for platelet function and ultrastructural characteristics, levels of prothrombotic factors, and cerebral morphology/metabolism/function at baseline, with consistent results in all the parameters analyzed.

### ITA Nearly Normalizes Glycometabolic Control

Mean duration of follow-up was 15.5 ± 2.5 months. Renal function, blood pressure, and lipid metabolism measurements (creatinine: T1D-WL 0.8 ± 0.1 vs. ITA 1.0 ± 0.2 mg/dL; total cholesterol: T1D-WL 172.5 ± 11.0 vs. ITA 183.5 ± 12.5 mg/dL; HDL cholesterol: T1D-WL 54.3 ± 10.5 vs. ITA 58.9 ± 9.9 mg/dL; triglycerides: T1D-WL 97.0 ± 37.7 vs. ITA 64.1 ± 18.8 mg/dL; and mean arterial pressure: T1D-WL 98.6 ± 8.1 vs. ITA 103.8 ± 1.8 mmHg) were similar at 15 months of follow-up in individuals with T1D. Patients did not show any sign of cardiovascular disease (absence of both any electrocardiographic signs of myocardial ischemia and any echocardiography signs of systolic/diastolic dysfunction). A significant improvement in glycometabolic control was evident in patients in the ITA group compared with individuals in the T1D-WL group. *S*-glucose, C-peptide, $HbA_{1c}$, and exogenous insulin requirement values were all improved in patients in the ITA group (Fig. 1*A–D*). Islet transplantation restored glycometabolic control to near normal.

### ITA Is Associated With Near-Normal Platelet Morphology, Aggregation, and Calcium Platelet Homeostasis

We examined an average of 10 platelets per patient to evaluate platelet area, calcium platelet homeostasis, and number of granules, and we examined 10 granules to evaluate granular area (Fig. 1*E* and *F*). Platelet size at 15 months of follow-up was higher in individuals with T1D compared with controls and ITA patients (T1D-WL 3.860 ± 0.288 × $10^6$, CTRL 3.076 ± 0.197 × $10^6$, and ITA 3.199 ± 0.287 × $10^6$ $nm^2$; $P = 0.02$, T1D-WL vs. CTRL), whereas ITA patients and controls showed similar platelet sizes ($P$ = not significant). Moreover, platelets in individuals with T1D showed the tendency to aggregate, exhibited a more electron-dense cytoplasm, and displayed more numerous and larger granules than platelets in controls and the ITA patients (Fig. 1*G*). This suggests that platelet morphology is altered in

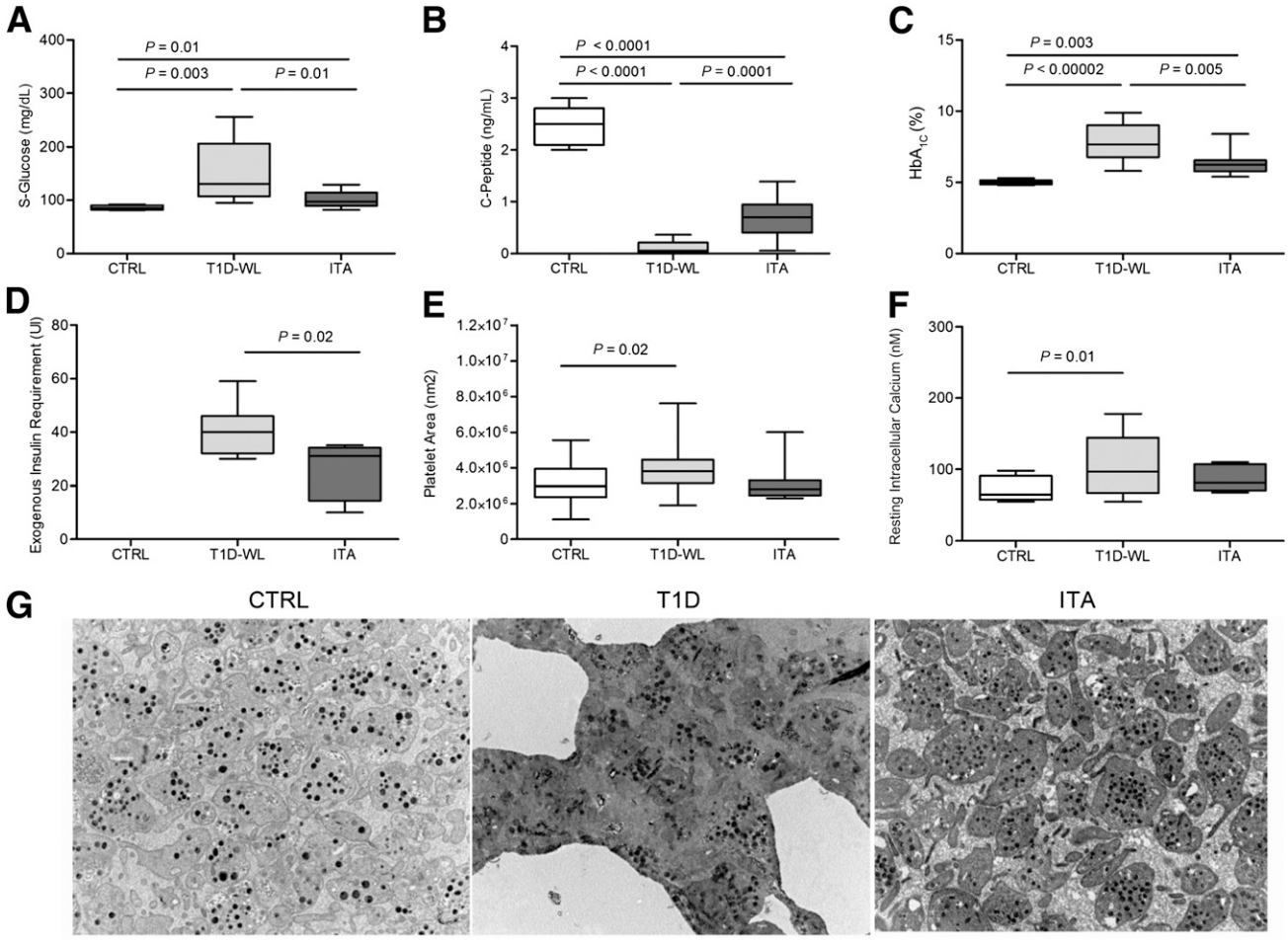



**Figure 1—**Metabolic parameters, resting calcium, and platelet morphology in patients who underwent ITA, in individuals with T1D-WL, and in healthy controls (CTRL) at 15 months of follow-up. Serum glucose (*A*), C-peptide (*B*), $HbA_{1c}$ (*C*), and exogenous insulin requirement (*D*) were improved in patients who underwent ITA compared with the T1D-WL group. Platelet areas were also higher in the T1D-WL compared with the CTRL group ($P = 0.02$) (*E*). *F*: Resting $[Ca^{2+}]i$ was significantly higher in the T1D-WL group than in ITA and CTRL groups ($P = 0.01$). Electron microscopy revealed that in the CTRL group (*G*) platelets show secretory granules (primarily α-type), glycogen, and several tubules and vesicles. Platelets from individuals with T1D displayed a tendency to aggregate, and their cytoplasm was denser than platelets in the CTRL and ITA groups (*G*).

individuals with T1D and that islet transplantation is associated with near-normalization of platelet size and aggregation and platelet granule size and number. The levels of resting $[Ca^{2+}]i$, analyzed at 15 months of follow-up, were higher in individuals with T1D, but not in ITA patients, compared with controls (CTRL 72.2 ± 15.0, T1D-WL 107.7 ± 40.0, ITA 87.6 ± 18.8 nmol; $P$ = 0.01) (Fig. 1*F*). This suggests that T1D is associated with aberrant calcium platelet homeostasis and that islet transplantation promotes near-normalization of both platelet signaling and the cytosolic calcium pathway.

### ITA Is Associated With Reduced Hemostatic Abnormalities

Analysis of hemostatic profile demonstrated high levels of plasmatic Fg, F1+2, and D-dimer in individuals with T1D. Lower levels of Fg (CTRL 262.5 ± 43.0, T1D-WL 328.5 ± 35.0, ITA 367.0 ± 26.0 mg/dL; $P < 0.005$, CTRL vs. all) and F1+2 (CTRL 63 ± 17.8, T1D-WL 113 ± 35.7, ITA 96 ± 45.2 mmol/L; $P$ = 0.005, CTRL vs. T1D-WL) were evident in controls compared with ITA patients and individuals with T1D (Fig. 2*A* and *B*). Finally, D-dimer levels were higher in individuals with T1D compared with controls and ITA patients (CTRL 0.19 ± 0.02, T1D-WL 1.07 ± 0.80, ITA 0.24 ± 0.02 μg/mL; $P$ = 0.01, CTRL vs. all) (Fig. 2*C*). No statistically significant differences were found in fasting homocysteine levels among the three groups of subjects (Fig. 2*D*). Analysis of prothrombin time, activated partial thromboplastin time, and antithrombin III did not reveal differences among the three groups at 15 months of follow-up. Significantly lower levels of protein S and protein C were found in individuals with T1D compared with controls, whereas in ITA patients levels were almost normalized at the 15-month follow-up (Figs. 2*E* and *F*).

A prothrombotic state is evident in individuals with T1D, and ITA is associated with near-normalization of hemostatic abnormalities.

### ITA Does Not Alter Cerebral Morphology: A Conventional MRI Study

Evaluation of vasculopathy with conventional MRI revealed that 12 of 22 individuals with T1D had cerebrovascular disease at baseline, without differences between the two groups (T1D-ITA vs. T1D-WL; $P$ = not significant), compared with 2 of 9 controls (T1D 54.5% [95% CI 36–69%] vs. CTRL 22.2% [95% CI 3–6]; $P$ = 0.04).

No major changes in cerebral morphology were evident at 15 months of follow-up. Mean cerebral volume was similar in individuals with T1D and in ITA patients, whereas it was significantly lower when compared with controls at 15 months of follow-up (CTRL 1.197 ± 98.3, ITA 1.027 ± 90.3, T1D-WL 1.105 ± 67.8; $P < 0.03$, CTRL vs. all) (Fig. 3*A*). Islet transplantation did not modify structural changes in cerebral morphology, which was evident in individuals with T1D at 15 months of follow-up.

### ITA Is Associated With Improved Cerebral Metabolism: An MRS Study

#### *Nonlocalized MRS*

The mean absolute NAA, a neuronal marker that indicates neuronal/axonal loss when decreased (29,35), showed similar values in the three groups of patients at 15 months of follow-up. Absolute NAA values, corrected for brain volume to adjust for interindividual variation (NAA concentration), failed to reveal any effect in the ITA group (Fig. 3*B*). Likewise, mean whole-brain NAA content did not differ among the three groups (CTRL 14.5 ± 1.5; T1D 14.4 ± 1.8; ITA 13.2 ± 1.6 arbitrary units [Aus]; $P$ = not significant) (Fig. 3*C*).

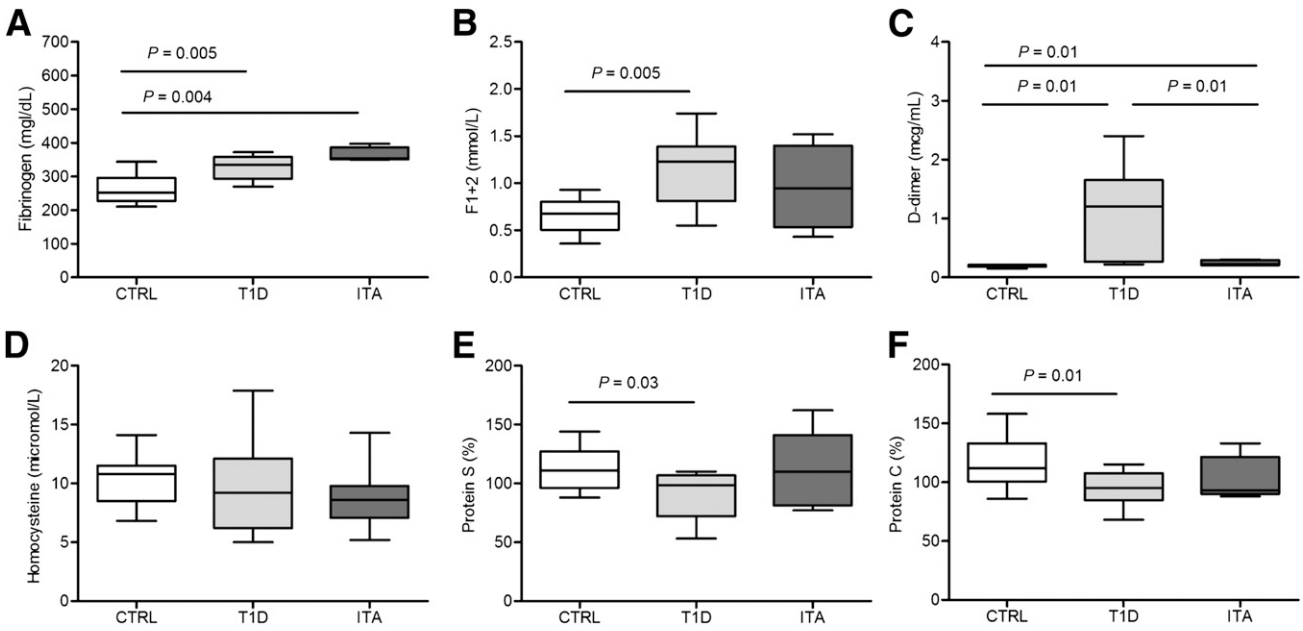



**Figure 2**—Hypercoagulability markers in patients who underwent ITA, in individuals with T1D-WL, and in healthy controls (CTRL) at 15 months of follow-up. Fibrinogen ($P$ = 0.005) (*A*), F1+2 ($P$ = 0.005) (*B*), and D-dimer levels ($P$ = 0.01) (*C*) were higher in the T1D-WL group compared with the CTRL group ($P$ = 0.01) (*C*). No differences were found among groups for fasting homocysteine levels (*D*). Protein S and C activity were lower in the T1D-WL group compared with the CTRL group, and a near-normalization of their levels was evident in the ITA group (protein S: CTRL vs. T1D-WL, $P$ = 0.03 [*E*]; protein C: CTRL vs. T1D-WL, $P$ = 0.01 [*F*]).

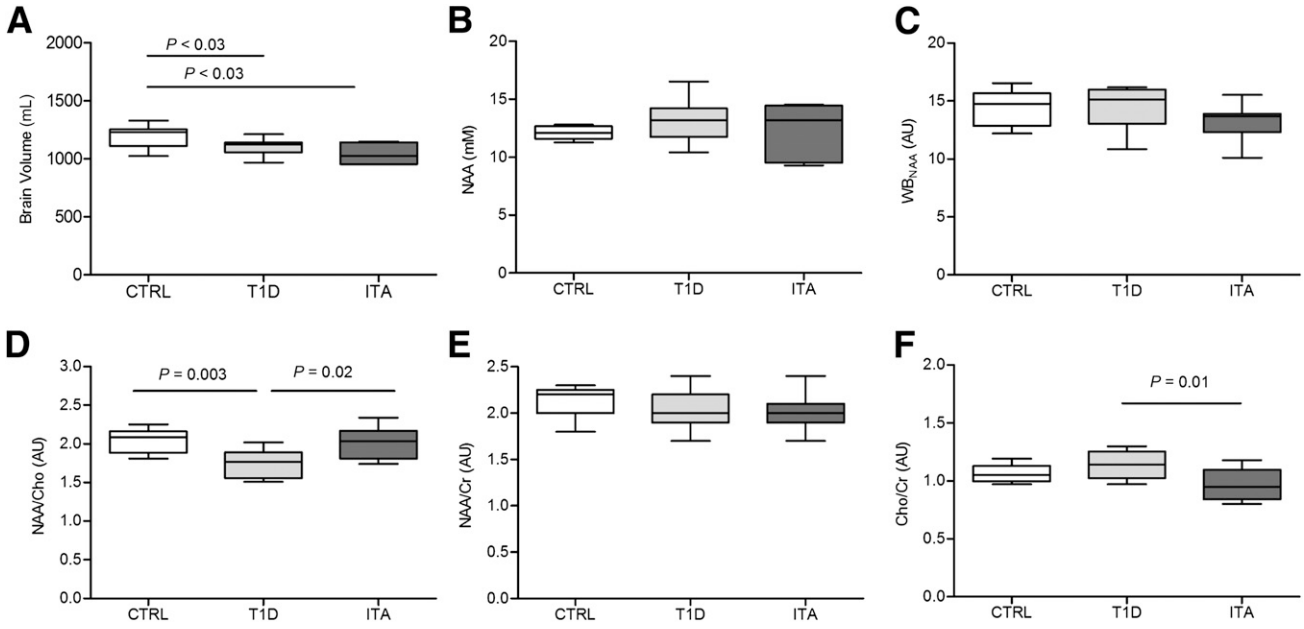



**Figure 3—**Conventional MRI imaging and $^{1}$H-MRS analysis in patients who underwent ITA, in individuals with T1D, and in healthy controls (CTRL) at 15 months of follow-up. Brain volume (*A*) was higher in the CTRL group compared with the ITA and T1D groups. No other differences between groups were evident in NAA (*B*), whole-brain NAA (*C*), and NAA/Cr (*E*). NAA/Cho (*D*) was significantly lower in individuals with T1D on the waiting list (T1D-WL) compared with controls, whereas it was near-normal in patients undergoing ITA. The Cho-to-Cr ratio was increased in the T1D-WL group compared with both the ITA and CTRL groups, suggesting an increase in degenerative processes ($P = 0.01$) (*F*).

***Localized MRS Single-Voxel Spectroscopy***

The NAA-to-Cho ratio (an index of neuroaxonal loss/impairment associated with diabetes-related gliosis [36]) was similar in ITA patients and controls, but it was lower in individuals with T1D, suggesting major cerebral tissue damage (CTRL 2.0 ± 0.2, T1D-WL 1.7 ± 0.2, ITA 1.9 ± 0.2 AU; $P = 0.02$, T1D-WL vs. ITA; $P = 0.003$, CTRL vs. T1D-WL), (Fig. 3*D*). The NAA-to-Cr ratio, an index of neuronal loss/damage when decreased (37), did not show any significant difference in the three groups analyzed, although higher values were found in controls compared with others (CTRL 2.1 ± 0.2, T1D-WL 2.0 ± 0.1, ITA 2.0 ± 0.2 AU; *P* = not significant) (Fig. 3*E*). Conversely, we documented an increased Cho-to-Cr ratio (which has been associated with diabetes gliosis [36] when increased) in individuals with T1D, particularly when compared with ITA patients, indicating a near recovery of the neuronal tissue (T1D-WL 1.1 ± 0.1 and ITA 0.98 ± 0.1; $P = 0.01$) (Fig. 3*F*). Finally, an inverse linear correlation was found between $HbA_{1c}$ and the NAA-to-Cho ratio ($r = -0.61$; $P = 0.004$) (data not shown), thus suggesting that poor glycemic control in individuals with T1D is important in promoting degenerative phenomena and altering cerebral metabolism.

**ITA Is Associated With Improved Emotional Status: A Psychological and Neuropsychological Assessment**

Psychological evaluation was performed by analyzing patients' emotional axis and QoL at 15 months' follow-up using the POMS test and LEIPAD questionnaire, respectively (30,31). The POMS test, examining six different dimensions of mood (see RESEARCH DESIGN AND METHODS), showed a significantly higher score in the POMS depression-dejection category among individuals with T1D compared with controls and ITA patients (Table 1). Moreover, the POMS fatigue-inertia category was significantly different among controls, individuals with T1D, and ITA patients, with the latter showing less fatigue-inertia (Table 1). Results of the POMS confusion-bewilderment category showed higher values in individuals with T1D compared with ITA patients, whereas the POMS anger-hostility category revealed a significant difference in controls compared with both individuals with T1D and ITA patients (Table 1). No differences were found in the POMS tension-anxiety and vigor-activity categories (Table 1). The analysis of the LEIPAD test did not show any difference in physical function and self care among the three groups, but it revealed a significantly higher score for depression and anxiety in individuals with T1D compared with ITA patients (Table 1). In addition, life satisfaction scores were significantly lower (in which lower score demonstrates improvement) in controls and ITA patients compared with individuals with T1D (Table 1). Considering both the POMS test and LEIPAD questionnaire, despite the fact that some values did not achieve statistical significance, an overall trend demonstrating improvement was evident in ITA patients compared with individuals with T1D. This was confirmed by neuropsychological assessment, in which ITA patients demonstrated a substantial benefit from transplantation compared with individuals with T1D in most of the tests (Table 1). Phonemic and semantic fluency also showed higher scores in controls and ITA patients compared with individuals with T1D. Furthermore, the paced auditory serial addition test revealed significantly higher scores among ITA patients

**Table 1—Psychological and neuropsychological evaluations in patients with ITA and those with T1D-WL***

| Evaluations | CTRL ($n$ = 10) | T1D-WL ($n$ = 10) | ITA ($n$ = 12) | $P$ values: CTRL vs. ITA | $P$ values: CTRL vs. T1D-WL | $P$ values: T1D-WL vs. ITA |
|---|---|---|---|---|---|---|
| POMS | | | | | | |
| Tension/anxiety | 10.8 ± 1.3 | 11.6 ± 6.2 | 9.6 ± 2.7 | 0.38 | 0.81 | 0.28 |
| Depression/dejection | 8.6 ± 0.9 | 9.2 ± 5.6 | 2.6 ± 2.4 | 0.0002 | 0.76 | 0.02 |
| Anger/hostility | 10.2 ± 2.5 | 5.2 ± 4.0 | 5.8 ± 3.5 | 0.03 | 0.01 | 0.80 |
| Vigor/activity | 14.0 ± 3.1 | 13.9 ± 7.8 | 17.6 ± 5.8 | 0.38 | 0.97 | 0.22 |
| Fatigue/inertia | 9.0 ± 2.2 | 5.5 ± 3.5 | 4.6 ± 2.5 | 0.008 | 0.04 | 0.60 |
| Confusion/bewilderment | 4.8 ± 1.1 | 8.2 ± 3.9 | 4.3 ± 2.0 | 0.6 | 0.08 | 0.04 |
| Physical function | 2.4 ± 1.1 | 4.3 ± 2.2 | 2.8 ± 0.4 | 0.14 | 0.48 | 0.09 |
| Self-care | 0.2 ± 0.4 | 0.8 ± 1.7 | 0.0 ± 0.0 | 0.33 | 0.14 | 0.64 |
| Depression and anxiety | 2.0 ± 0.7 | 3.5 ± 1.9 | 1.2 ± 0.8 | 0.12 | 0.14 | 0.02 |
| Cognitive functioning | 2.2 ± 0.4 | 3.9 ± 2.2 | 3.0 ± 0.7 | 0.41 | 0.07 | 0.13 |
| Social functioning | 2.4 ± 1.1 | 3.3 ± 2.5 | 3.8 ± 1.9 | 0.73 | 0.30 | 0.55 |
| Sexual functioning | 1.8 ± 0.8 | 2.2 ± 1.9 | 2.4 ± 0.8 | 0.85 | 0.30 | 0.65 |
| Life satisfaction | 2.4 ± 0.5 | 7.3 ± 2.3 | 5.5 ± 3.2 | 0.05 | 0.0006 | 0.22 |
| MMSE | 29.0 ± 0.7 | 29.1 ± 1.2 | 28.8 ± 1.3 | 0.67 | 0.82 | 0.90 |
| Token | 33.4 ± 0.8 | 33.6 ± 1.9 | 33.0 ± 1.8 | 0.56 | 0.83 | 0.75 |
| Phonemic fluency | 38.6 ± 6.5 | 30.5 ± 9.9 | 33.4 ± 8.8 | 0.13 | 0.03 | 0.6 |
| Semantic fluency | 49.2 ± 7.3 | 39.1 ± 10.0 | 46.8 ± 9.6 | 0.50 | 0.03 | 0.12 |
| Long-term verbal memory | 16.8 ± 1.9 | 14.2 ± 4.6 | 14.8 ± 4.3 | 0.81 | 0.37 | 0.22 |
| Short-term verbal memory | 4.9 ± 0.5 | 5.3 ± 1.1 | 5.3 ± 0.7 | 0.89 | 0.40 | 0.44 |
| Corsi | 5.1 ± 0.8 | 4.5 ± 0.6 | 4.8 ± 0.5 | 0.42 | 0.44 | 0.17 |
| PASAT | 41.4 ± 9.1 | 36.8 ± 14.8 | 49.5 ± 7.2 | 0.05 | 0.50 | 0.04 |
| Stroop test | | | | | | |
| Color naming | 14.0 ± 3.4 | 21.7 ± 4.7 | 23.0 ± 3.8 | 0.01 | 0.02 | 0.65 |
| Word-color interference | 17.2 ± 5.5 | 35.7 ± 5.6 | 32.5 ± 6.1 | 0.003 | 0.0001 | 0.41 |
| Color-color interference | 36.8 ± 21.4 | 46.8 ± 4.4 | 43.2 ± 2.3 | 0.16 | 0.55 | 0.24 |
| Stroop error | 0.6 ± 1.3 | 3.8 ± 3.4 | 4.1 ± 3.4 | 0.03 | 0.87 | 0.02 |
| WCST | 6.0 ± 0.0 | 5.9 ± 0.3 | 6.0 ± 0.0 | 0.89 | 0.74 | 0.49 |

Data are mean ± SD. MMSE, Mini-Mental State Examination; PASAT, Paced Auditory Serial Addition Test; WCST, Wisconsin Card Sorting Test.
*Psychological evaluation: profile of mood state analysis of depression-dejection and fatigue-inertia tracts, as well as evaluation of physical function showed better results in ITA compared with individuals with T1D-WL. Neuropsychological evaluation: phonemic word fluency, paced auditory serial addition test, and Stroop tests are near-normalized in the ITA group compared with the T1D-WL group at 15 months of follow-up.

compared with individuals with T1D and controls (Table 1). The color-naming and the word-color interference variants of the Stroop test were higher (indicating a worsened state) in T1D-WL and ITA patients compared with controls (Table 1). The color-color interference Stroop test revealed lower scores (i.e., a worse state) in T1D-WL and ITA patients compared with controls, although this difference did not reach statistical significance. The number of errors made by the patients during the three sections of the Stroop test was significantly lower in controls than in T1D-WL and ITA patients (Table 1). ITA is associated with a near-normalization of psychological and neuropsychological tests.

### Immunosuppression Treatment Does Not Exert Any Beneficial Effect on Hemostatic Abnormalities in Islet Transplantation

To understand whether concomitant immunosuppressive treatment may have biased our results, we first explored the effect of immunosuppression (tacrolimus and sirolimus) in altering coagulatory/inflammatory markers in individuals with ESRD who had received a kidney transplant ($n$ = 10). We finally evaluated whether immunosuppression (sirolimus) altered coagulatory/inflammatory markers in individuals with T1D ($n$ = 5). Immunosuppression did not alter or change the peripheral levels of D-dimers, proteins S and C, homocysteine, Fg, or F1+2 in individuals who received a kidney transplant compared with individuals with ESRD (Supplementary Fig. 2*A*–*F*) or in patients with T1D (Supplementary Fig. 2*G*–*N*). These experiments confirm that immunosuppression has no effect on inflammatory/coagulatory markers, and thus it is not likely involved in the modifications of these markers observed in our study.

## CONCLUSIONS

Insulin, the only treatment so far for individuals with T1D, can only partially prevent long-term diabetes complications, and it is still associated

with fatal hypoglycemic episodes (4). Islet transplantation, a therapeutic option available for selected individuals with T1D, has been further examined in the past decade because it can potentially normalize glycometabolic control, at least in the short term, while providing partial islet function capable of halting the progression of diabetes complications (38,39). The safety, effectiveness, and minimal invasiveness of islet transplantation combined with kidney transplantation have been demonstrated by our group and others (4,5,40). Unfortunately, as with other allogeneic procedures, islet transplantation also requires administration of lifelong immunosuppressive treatment (7,41). Immunosuppressants (e.g., tacrolimus and sirolimus) increased the incidence of cardiovascular disease because of their detrimental effect on lipid metabolism and blood pressure (11), which may call into question the advantage of ITA in truly halting the progression of vascular complications associated with T1D. Our results confirmed that ITA is able to reestablish good glycemic control at 15 months of follow-up and maintain a stable lipid profile and acceptable blood pressure control, despite ongoing immunosuppressive treatment.

Furthermore, our analysis of the hemostasis/thrombosis system demonstrated that patients undergoing ITA had normal platelet volume/function and normal levels of prothrombotic factors. On the contrary, individuals with T1D who remained on the waiting list showed a persistent state of hypercoagulability, which represents a significant risk factor for vascular thrombosis and atherosclerosis in different systems, including the cerebral vascular system. In particular, activation of platelets strictly depends on cytosolic calcium signaling, which tends to be altered in T1D (24). Platelet activation, together with increased prothrombotic factors, leads to persistent activation of the clotting system and facilitates generation of thrombi and vessel occlusion.

We then used $^{1}$H-MRS to analyze brain metabolism and evaluate whether these hemostatic/platelet alterations resulted in or were associated with any modification of cerebral morphology, metabolism, and function (28). Amounts of total Cr (associated with energy metabolism and considered as an internal standard), compounds containing Cho (playing an important role in the turnover of cellular wall), and NAA (a marker of neuronal density and functioning) in the brain, the relative reductions of which have been correlated with neuronal/axonal defect in number and function (29,35), were measured. $^{1}$H-MRS showed specific alterations in cerebral metabolism, which were otherwise undetectable with standard methods, including signs of atrophy, decreased brain volume, and reduced axonal metabolism in individuals with T1D compared with healthy controls. These metabolic abnormalities can be associated with early cognitive decline and possibly senile dementia. Conversely, we observed higher NAA-to-Cho and lower Cho-to-Cr ratio values in patients undergoing ITA, indicating a relative sparing of neuronal function from tissue degeneration and loss after ITA (36,37). Finally, we documented a significant improvement in mood profile, depression and anxiety, speed of information processing, and attention abilities, with greater maintenance of neuropsychological attitude in patients receiving ITA compared with individuals with T1D. The presence of a positive correlation between $HbA_{1c}$ levels and the NAA-to-Cho ratio (a good marker of axonal/neuronal degeneration) demonstrated the importance of good metabolic control in patients receiving ITA (and overall in individuals with T1D) in influencing the preservation of neural structures and cognitive function/metabolism. We acknowledge that long-term immunosuppression is not an option for individuals with T1D, but our study is a proof of concept demonstrating that islet transplantation, despite immunosuppression, can prevent the occurrence of major diabetes complications.

### Limitations of the Study

Our study is not devoid of limitations. First, the sample size is relatively small, and a larger study is required to generalize conclusions. Second, although the two cohorts of individuals with T1D (T1D-WL and T1D-ITA) were homogeneous at baseline, we cannot completely exclude the presence of biases. In this context, we acknowledge the complexity of our whole clinical assessment, which will require further validation. Another bias may be represented by concomitant immunosuppression, which may affect coagulatory/inflammatory markers. Indeed, our data for individuals with ESRD who received a kidney transplant and for individuals with T1D treated with sirolimus demonstrated that immunosuppression has no effects on coagulatory/inflammatory markers—at least in our study—and thus should not be a confounding factor. Our results were obtained in a relative short period of time, underlining the relevance that ITA may have in halting diabetes complications in individuals with T1D (Supplementary Table 2). However, the field of islet transplantation needs randomized, well-designed, and powered studies to solidly confirm the benefits of this approach.In conclusion, the restoration of near-normal glucose metabolism with ITA improves glycometabolic control and hemostatic abnormalities, thereby leading to the near-normalization of cerebral disorders (Supplementary Table 2). Despite the disadvantageous effects of immunosuppressive therapy, islet transplantation may be an important option to limit the progression of major T1D complications but must to be validated in a larger, controlled clinical trial.

**Funding.** This work was supported by the Juvenile Diabetes Research Foundation (grant no. JT01Y01). F.D. has received an Italian Scientists and Scholars of North America Foundation–Fondazione Marche Fellowship. A.V. has received support from a National Institutes of Health Research Training Grant to Boston Children's Hospital in Pediatric Nephrology (T32DK-007726-28). R.B. has received support from an American Diabetes Association (ADA) mentor-based fellowship to P.F. P.F. is supported by a Translational Research Program grant from Boston Children's Hospital and has received a Juvenile Diabetes Research Foundation Career Development Award, an American Society of Nephrology Career Development Award, an ADA

mentor-based fellowship, Istituto Superiore di Sanitá grant ("Staminali" RF-FSR-2008-1213704), and Italian Ministry of Health grants RF 2010-2314794 and RF 2010-233119.

**Duality of Interest.** No potential conflicts of interest relevant to this article were reported.

**Author Contributions.** F.D. designed the study, performed the research, analyzed data, and wrote the article. P.M., G.F., and S.L.R. performed research and analyzed data. P.V., A.V., A.M., R.B., R.N., M.F., E.C., A.D., I.F., and A.F. performed research. F.P. designed the research and wrote and edited the paper. G.M. and L.P. edited the paper. F.F. and A.S. designed the study and edited the article. P.F. designed the study and wrote and edited the article. P.F. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Prior Presentation.** This study was presented in oral form at the 73rd Scientific Sessions of the American Diabetes Association, Chicago, Illinois, 21–25 June 2013.

## References

1. Nathan DM, Cleary PA, Backlund JY, et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med 2005;353:2643–2653
2. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977–986
3. Melendez-Ramirez LY, Richards RJ, Cefalu WT. Complications of type 1 diabetes. Endocrinol Metab Clin North Am 2010;39:625–640
4. Fiorina P, Shapiro AM, Ricordi C, Secchi A. The clinical impact of islet transplantation. Am J Transplant 2008;8:1990–1997
5. Pileggi A, Ricordi C, Kenyon NS, et al. Twenty years of clinical islet transplantation at the Diabetes Research Institute–University of Miami. Clin Transpl 2004:177–204
6. Tharavanij T, Betancourt A, Messinger S, et al. Improved long-term health-related quality of life after islet transplantation. Transplantation 2008;86:1161–1167
7. Shapiro AM, Ricordi C, Hering BJ, et al. International trial of the Edmonton protocol for islet transplantation. N Engl J Med 2006;355:1318–1330
8. Young JB, Neumayer HH, Gordon RD. Pretransplant cardiovascular evaluation and posttransplant cardiovascular risk. Kidney Int Suppl 2010;118:S1–S7
9. Jardine AG, Gaston RS, Fellstrom BC, Holdaas H. Prevention of cardiovascular disease in adult recipients of kidney transplants. Lancet 2011;378:1419–1427
10. Guerra G, Ciancio G, Gaynor JJ, Zarak A, Brown R, Hanson L, et al. Randomized trial of immunosuppressive regimens in renal transplantation. J Am Soc Nephrol 2011;22:1758–1768
11. Hricik DE. Metabolic syndrome in kidney transplantation: management of risk factors. Clin J Am Soc Nephrol 2011;6:1781–1785
12. Vincenti F, Friman S, Scheuermann E, et al. Results of an international, randomized trial comparing glucose metabolism disorders and outcome with cyclosporine versus tacrolimus. Am J Transplant 2007;7:1506–1514
13. Robertson RP. Islet transplantation a decade later and strategies for filling a half-full glass. Diabetes 2010;59:1285–1291
14. Fiorina P, Folli F, Zerbini G, et al. Islet transplantation is associated with improvement of renal function among uremic patients with type I diabetes mellitus and kidney transplants. J Am Soc Nephrol 2003;14:2150–2158
15. Senior PA, Zeman M, Paty BW, Ryan EA, Shapiro AM. Changes in renal function after clinical islet transplantation: four-year observational study. Am J Transplant 2007;7–:91–98
16. Fiorina P, Gremizzi C, Maffi P, et al. Islet transplantation is associated with an improvement of cardiovascular function in type 1 diabetic kidney transplant patients. Diabetes Care 2005;28:1358–1365
17. Venturini M, Fiorina P, Maffi P, et al. Early increase of retinal arterial and venous blood flow velocities at color Doppler imaging in brittle type 1 diabetes after islet transplant alone. Transplantation 2006;81:1274–1277
18. Danielson KK, Hatipoglu B, Kinzer K, Kaplan B, Martellotto J, Qi M, et al. Reduction in carotid intima-media thickness after pancreatic islet transplantation in patients with type 1 diabetes. Diabetes Care 2013;36:450–456
19. Cerbone AM, Macarone-Palmieri N, Saldalamacchia G, Coppola A, Di Minno G, Rivellese AA. Diabetes, vascular complications and antiplatelet therapy: open problems. Acta Diabetol 2009;46:253–261
20. Eckel RH, Wassef M, Chait A, Sobel B, Barrett E, King G, et al. Prevention Conference VI: Diabetes and Cardiovascular Disease: Writing Group II: pathogenesis of atherosclerosis in diabetes. Circulation 2002;105:e138–e143
21. Sima AA. Encephalopathies: the emerging diabetic complications. Acta Diabetol 2010;47:279–293
22. Franceschi M, Cecchetto R, Minicucci F, Smizne S, Baio G, Canal N. Cognitive processes in insulin-dependent diabetes. Diabetes Care 1984;7:228–231
23. Maffi P, Scavini M, Socci C, et al. Risks and benefits of transplantation in the cure of type 1 diabetes: whole pancreas versus islet transplantation. A single center study. Rev Diabet Stud 2011;8:44–50
24. Fiorina P, Folli F, D'Angelo A, et al. Normalization of multiple hemostatic abnormalities in uremic type 1 diabetic patients after kidney-pancreas transplantation. Diabetes 2004;53:2291–2300
25. Rink TJ, Sage SO. Calcium signaling in human platelets. Annu Rev Physiol 1990;52:431–449
26. O'Brien JT, Paling S, Barber R, et al. Progressive brain atrophy on serial MRI in dementia with Lewy bodies, AD, and vascular dementia. Neurology 2001;56:1386–1388
27. Rovaris M, Filippi M, Calori G, et al. Intra-observer reproducibility in measuring new putative MR markers of demyelination and axonal loss in multiple sclerosis: a comparison with conventional T2-weighted images. J Neurol 1997;244:266–270
28. Fiorina P, Vezzulli P, Bassi R, Gremizzi C, Falautano M, D'Addio F, et al. Near normalization of metabolic and functional features of the central nervous system in type 1 diabetic patients with end-stage renal disease after kidney-pancreas transplantation. Diabetes Care 2012;35:367–374
29. Miller BL. A review of chemical issues in 1H NMR spectroscopy: N-acetyl-L-aspartate, creatine and choline. NMR Biomed 1991;4:47–52
30. Albrecht RR, Ewing SJ. Standardizing the administration of the Profile of Mood States (POMS): development of alternative word lists. J Pers Assess 1989;53:31–39
31. De Leo D, Diekstra RF, Lonnqvist J, et al. LEIPAD, an internationally applicable instrument to assess quality of life in the elderly. Behav Med 1998;24:17–27
32. De Renzi E, Vignolo LA. The token test: A sensitive test to detect receptive disturbances in aphasics. Brain 1962;85:665–678
33. Tombaugh TN. A comprehensive review of the Paced Auditory Serial Addition Test (PASAT). Arch Clin Neuropsychol 2006;21:53–76
34. Feldstein SN, Keller FR, Portman RE, Durham RL, Klebe KJ, Davis HP. A comparison of computerized and standard versions of the Wisconsin Card Sorting Test. Clin Neuropsychol 1999;13:303–313
35. Ross B, Michaelis T. Clinical applications of magnetic resonance spectroscopy. Magn Reson Q 1994;10:191–247
36. Hershey T, Perantie DC, Wu J, Weaver PM, Black KJ, White NH. Hippocampal volumes

in youth with type 1 diabetes. Diabetes 2010;59:236–241

37. Lai PH, Chen PC, Chang MH, et al. In vivo proton MR spectroscopy of chorea-ballismus in diabetes mellitus. Neuroradiology 2001;43:525–531
38. Bassi R, Fiorina P. Impact of islet transplantation on diabetes complications and quality of life. Curr Diab Rep 2011;11:355–363
39. Poggioli R, Faradji RN, Ponte G, Betancourt A, Messinger S, Baidal DA, et al. Quality of life after islet transplantation. Am J Transplant 2006;6:371–378
40. Ryan EA, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, et al. Five-year follow-up after clinical islet transplantation. Diabetes 2005;54:2060–2069
41. Maffi P, Bertuzzi F, De Taddeo F, et al. Kidney function after islet transplant alone in type 1 diabetes: impact of immunosuppressive therapy on progression of diabetic nephropathy. Diabetes Care 2007;30:1150–1155

Copyright of Diabetes Care is the property of American Diabetes Association and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.