Altered Kidney Graft High-Energy Phosphate Metabolism in Kidney-Transplanted End-Stage Renal Disease Type 1 Diabetic Patients

A cross-sectional analysis of the effect of kidney alone and kidney-pancreas transplantation

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OBJECTIVE — Diabetes, hypertension, dyslipidemia, obesity, nephrotoxicity of certain immunosuppressive drugs, and the persistence of a chronic alloimmune response may significantly affect graft survival in end-stage renal disease (ESRD) type 1 diabetic patients who have undergone kidney transplant. The aim of this study was to ascertain the impact of kidney alone (KD) or combined kidney-pancreas (KP) transplantation on renal energy metabolism.

RESEARCH DESIGN AND METHODS — We assessed high-energy phosphates (HEPs) metabolism by using, in a cross-sectional fashion, ^{31}P-magnetic resonance spectroscopy in the graft of ESRD type 1 diabetic transplanted patients who received KD ($n = 20$) or KP ($n = 20$) transplant long before the appearance of overt chronic allograft nephropathy (CAN). Ten nondiabetic microalbuminuric kidney transplanted patients and 10 nondiabetic kidney transplanted patients with overt CAN were chosen as controls subjects.

RESULTS — Simultaneous KP transplantation patients showed a higher β-ATP/inorganic phosphorus (Pi) ratio (marker of the graft energy status) versus the other groups, and a positive correlation between β-ATP/Pi phosphorus ratio and A1C was found. In the analysis limited to the subgroup of normoalbuminuric patients, the difference in β-ATP/Pi was still detectable in KP patients compared with KD transplantation.

CONCLUSIONS — KP transplantation was associated with better HEPs than in KD transplantation, suggesting that restoration of β-cell function positively affects kidney graft metabolism.

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Abbreviations: ^{31}P-MRS, ^{31}P-magnetic resonance spectroscope; CAN, chronic allograft nephropathy; ESRD, end-stage renal disease; HEP, high-energy phosphate; KA, microalbuminuric kidney; KD, kidney alone; KP, kidney-pancreas; MRS, magnetic resonance spectroscopy; Pi, inorganic phosphorus; UAE, urinary albumin excretion.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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In type 1 diabetic and end-stage renal disease (ESRD) patients who have undergone kidney alone (KD) transplantation, diabetes, hypertension, dyslipidemia, obesity, nephrotoxicity of certain immunosuppressors, and the persistence of a chronic alloimmune response may significantly affect graft survival (1–3). Many of these factors can induce glomerulosclerosis, tubular damage, endothelial damage, and, ultimately, deterioration of renal function leading to the appearance of chronic allograft nephropathy (CAN) of the transplanted kidney (4).

Chronic allograft dysfunction is still one of the major risk factors that affect kidney survival, along with the death of the functioning graft (5). No major breakthroughs have been proposed in the last years, either in terms of early diagnosis or as far as new treatments; only the correction of the associated disorders has been pointed out (5). Other crucial points are *1*) the earlier the diagnosis of renal dysfunction, the more successful is the treatment and *2*) the degree of invasiveness of the approach is the other limiting factor.

Many authors strongly believe that ^{31}P-magnetic resonance spectroscopy (^{31}P-MRS) can provide a potential solution for such an important issue (6–8). Some previous articles (9,10) demonstrated that CAN is accompanied by a decrease in β-ATP/inorganic phosphorus (Pi) ratio at ^{31}P-MRS, and this was thought to be the result of chronic ischemia of the kidney graft, which causes a drop in ATP. In a recent study, Kazuhiko et al. (10) have demonstrated that a kidney β-ATP/Pi ratio at ^{31}P-MRS <1.2 is an independent risk factor for graft survival. They discussed the role of β-ATP/Pi ratio as a parameter for predicting long-term survival of a transplanted kidney, where a value >1.2 suggests a high probability of 3-year renal survival and a value >2.5 in-

Table 1—*Pre- and posttransplant characteristics of ESRD type 1 diabetic patients who underwent KP or KD transplant*

	KP	KD	KA	REJ
n	20	20	10	10
Age (years)	39.0 ± 1.7	42.3 ± 1.9	44.0 ± 4.8	39.8 ± 3.3
Diabetes duration (years)*	24.7 ± 1.5	27.3 ± 2.9	0	0
Dialysis duration (years)	2.7 ± 0.5	3.1 ± 0.5	4.0 ± 1.3	2.3 ± 0.6
Transplant follow-up (years)*	3.1 ± 0.5	4.2 ± 1.2	5.8 ± 1.0	7.1 ± 2.5
A1C (%)*	5.5 ± 0.1	8.7 ± 0.3	5.3 ± 0.1	5.5 ± 1.0
Fasting glucose levels (mg/dl)*	87.4 ± 4.0	171.1 ± 16.1	109.5 ± 5.0	111.5 ± 57.2
Cyclosporine trough levels (ng/ml)	176.2 ± 25.8	181.7 ± 10.1	165.5 ± 18.5	NA
Total cholesterol (mg/dl)	175.5 ± 10.5	178.7 ± 12.5	189.0 ± 16.0	161.6 ± 22.5
Triglycerides (mg/dl)*	93.4 ± 8.2	151.8 ± 18.1	133.0 ± 30.0	169.6 ± 29.3
HDL (mg/dl)*	53.1 ± 2.3	51.3 ± 2.9	42.2 ± 3.2	39.7 ± 4.1
Smoking habits	3:20	2:20	2:10	2:10
Hypertension	8:20	11:20	3:10	10:10
Systolic blood pressure (mmHg)*	134.3 ± 3.5	137.4 ± 4.7	136.2 ± 4.1	141.2 ± 5.4
Diastolic blood pressure (mmHg)*	81.5 ± 3.3	84.5 ± 323	83.1 ± 2.2	90.1 ± 4.1

Data are means ± SE or ratio. Two groups of patients with different renal function were chosen as controls (KA and REJ). *$P < 0.05$ between groups.

dicates that the transplanted kidney could survive >5 years (10).

There are emerging data that restoring β-cell function associated with the withdrawal of uremia in ESRD type 1 diabetes can prolong graft life compared with withdrawal of uremia alone (11–13).

The aim of our study was to evaluate whether metabolic markers of early kidney dysfunction were noninvasively detectable in vivo in the transplanted patients long before the appearance of overt CAN by using ^{31}P-MRS. Furthermore, we evaluated whether kidney-pancreas (KP) transplant confers a protection on the kidney graft in terms of high-energy phosphate (HEP) metabolism, particularly at an early stage. Finally, we used early markers of renal hemodynamic changes to asses the ability of ^{31}P-MRS to detect early alterations of kidney function (14,15).

RESEARCH DESIGN AND METHODS

Patients and transplantation

Forty ESRD type 1 diabetic patients were considered in our study and were enrolled in our waiting list for combined KP transplantation. A first group of patients underwent combined simultaneous KP transplantation ($n = 20$) from cadaver donors. The second group underwent KD transplantation ($n = 20$) and comprised either patients on the KP waiting list who received a kidney and a pancreas from a cadaver donor and who lost the pancreas graft early in the postoperative period (within 1–2 weeks, $n = 12$) due to pancreas thrombosis or patients who underwent renal transplantation only because the pancreas showed macroscopic damages at harvesting ($n = 8$). The two groups are comparable for the most important demographic characteristics upon enrollment in the study (Table 1).

Ten nondiabetic ESRD patients who received a kidney transplant and who experienced CAN (group REJ) and restarted hemodialysis and 10 nondiabetic ESRD patients who received kidney transplant and have microalbuminuria were chosen as controls for different degrees of renal dysfunction.

Immunosuppression

Organs for transplantation were obtained from cadaver donors through Nord Italia Transplant. All patients received the following immunosuppressive treatments: antithymoglobulin (IMTIX, SANGSTAT), cyclosporine (blood trough levels between 100 and 250 ng/ml), FK506 (blood levels between 10 and 15 ng/ml; only two patients in the KP group and two in the KD group were on FK506), mofetil mycophenolate (0.5–2 mg/day), and prednisone (5–10 mg/day). Steroids were tapered and then withdrawn within 6 months from time of transplant.

Study design and laboratory assessment

For this study, only patients with transplant follow-up >1 year were selected. Only KP and KD transplantation patients with good kidney graft function were included in the study. Other than the usual routine exams, urinary albumin excretion (UAE) was assessed in an early-morning spot urine sample (Albustix; Ames, Bayer Diagnostic, Bayer, Germany). In Albustix-positive samples, the urinary albumin concentration was measured by immunonephelometry with N albumin kits (Behring, Somerville, NY). A ratio between 17 and 299 was defined as microalbuminuria, while higher values were defined as overt macroalbuminuria.

Enzyme-linked immunosorbent assay for urinary thromboxane B2 and 6-keto-prostaglandin-F-1α levels

At the time of ^{31}P-MRS, we cross-sectionally analyzed urinary thromboxane B2 and 6-keto-prostaglandin-F-1α levels as markers of early deterioration of glomerular function (16). Urinary levels of 6-keto-prostaglandin-F-1α were assayed with a correlate enzyme immunosorbent assay kit (Assay Designs, Ann Arbor, MI). Urinary levels of thromboxane B2 were assayed with a correlate enzyme immunosorbent assay kit

^{31}P-MRS protocol

Renal ^{31}P-MRS was performed in the supine position with the use of a 1.5T whole-body scanner (Gyroscan Intera Master 1.5 MR System; Philips Medical Systems, Best, the Netherlands) as suggested by Klemm et al. (6). ^{31}P spectra were obtained by means of a 10-cm diameter surface coil used for transmission and detection of radio frequency signals at the resonance frequency of ^{31}P (at 1.5 T,

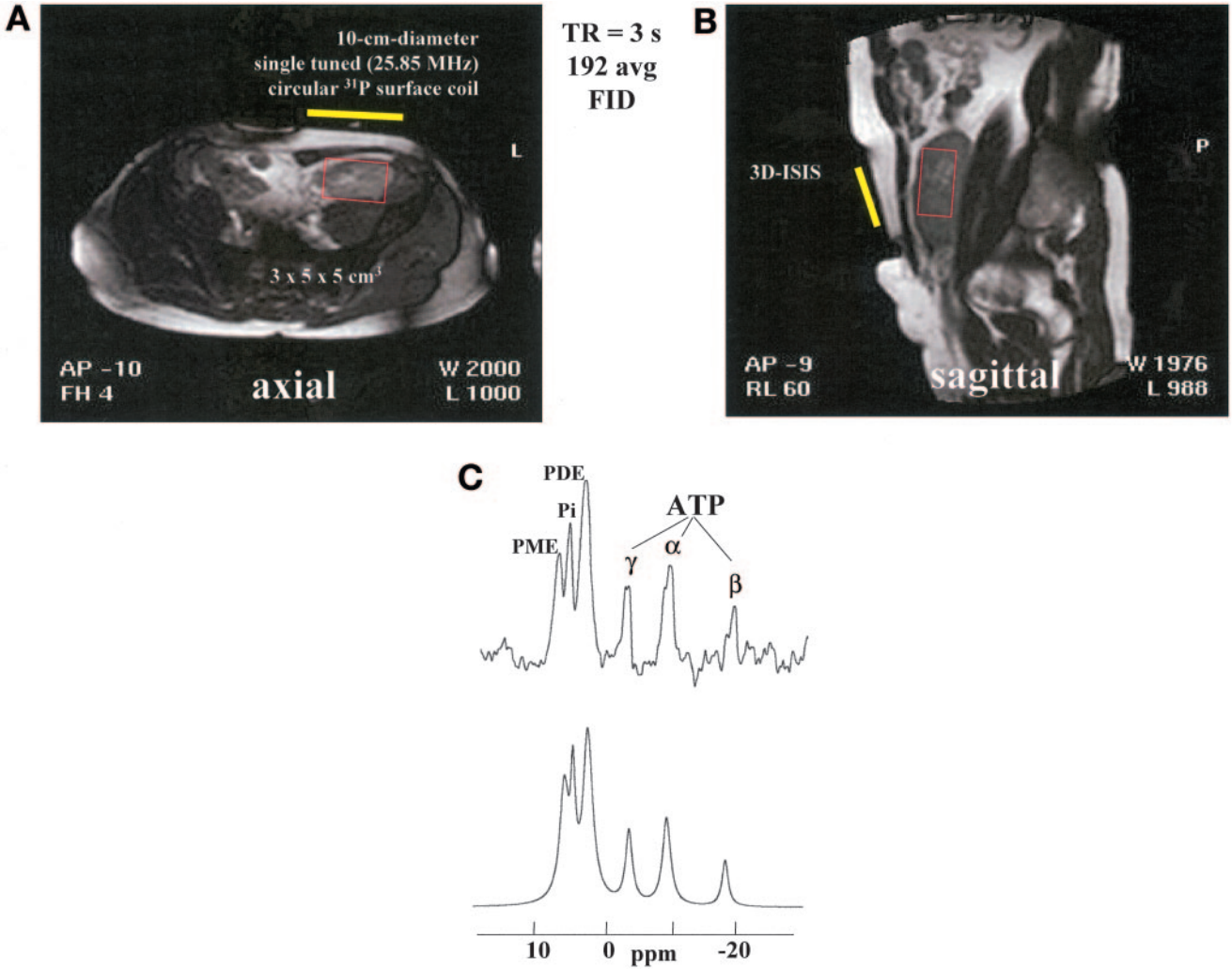

Figure 1—*Magnetic resonance imaging of a transplanted kidney graft shows the position of the volume of interest used to obtain the ^{31}P spectrum for both axial* (A) *and sagittal* (B) *positions. ^{31}P spectrum of a transplanted kidney* (C). *In the* lower panel, *the ^{31}P spectrum shows resonance peaks of phosphomonoesters (PME), Pi, phosphodiesters (PDE), and ATP. In the* upper panel, *the spectrum after line-fitting procedures is shown. FID, free induction decays; ISIS, image-selective in vivo spectroscopy; TR, repetion time.*

25.85 MHz). A small sample container built in the coil center, containing an aqueous solution of methyl-phosphonate, served as the geometrical reference. Standard imaging was performed to acquire scout images (SURVEY/BTFE) as shown in Fig. 1*A* and *B*. Shimming was performed with an automatic procedure by optimizing the ^{1}H-MR spectroscopy water signal obtained with a volume-selective 90°-180°-180° sequence. Shim volumes were planned on the transverse and sagittal scout images to include the entire kidney, while avoiding skin, muscles, and adipose tissue as much as possible. The transmitter receiver was then switched without time delay to the ^{31}P frequency. Manual tuning and matching of the ^{31}P surface coil were performed to adjust for different coil loading. The radio frequency level was adjusted to obtain a 180° pulse of 40 μs for the reference sample at the center of the ^{31}P surface coil. ISIS volume selection in three dimensions was the employed volume selection, based on 192 averaged free induction decays. The selection of the volume of interest was planned from the transverse and sagittal scout images and was oriented perpendicular to the abdomen-pelvic wall, avoiding inclusion of skin, adipose tissue, and muscles. Acquisition time was 9 min 48 s. Examination time was 30 min.

^{31}P-MR spectra were transferred to a remote SUN-SPARC workstation for analysis. The spectra were quantified automatically by model function analysis in the time domain. Signals were obtained also from phosphomonoesters, Pi, and phosphodiesters. The spectral fitting routine was based on a nonlinear least-squares Gauss-Newton implementation for exponential damping (Fig. 1*C*). The following parameters were obtained: β-ATP/Pi ratio (used as a marker of HEP metabolism in vivo), phosphmonoester, phosphodiester, α-ATP, β-ATP, and γ-ATP (10).

Statistical analysis

All the data were expressed as means ± SD. When the three groups were compared cross-sectionally, ANOVA with Tukey's test for multiple comparisons (for parametric data) or a Kruskal-Wallis test (for nonparametric data) was used according to distribution. Correlations were assessed with a Spearman's rank correlation coefficient. A P value <0.05 (by two-tailed testing) was considered an indicator of statistical significance. Analysis of data were done using SPSS statis-

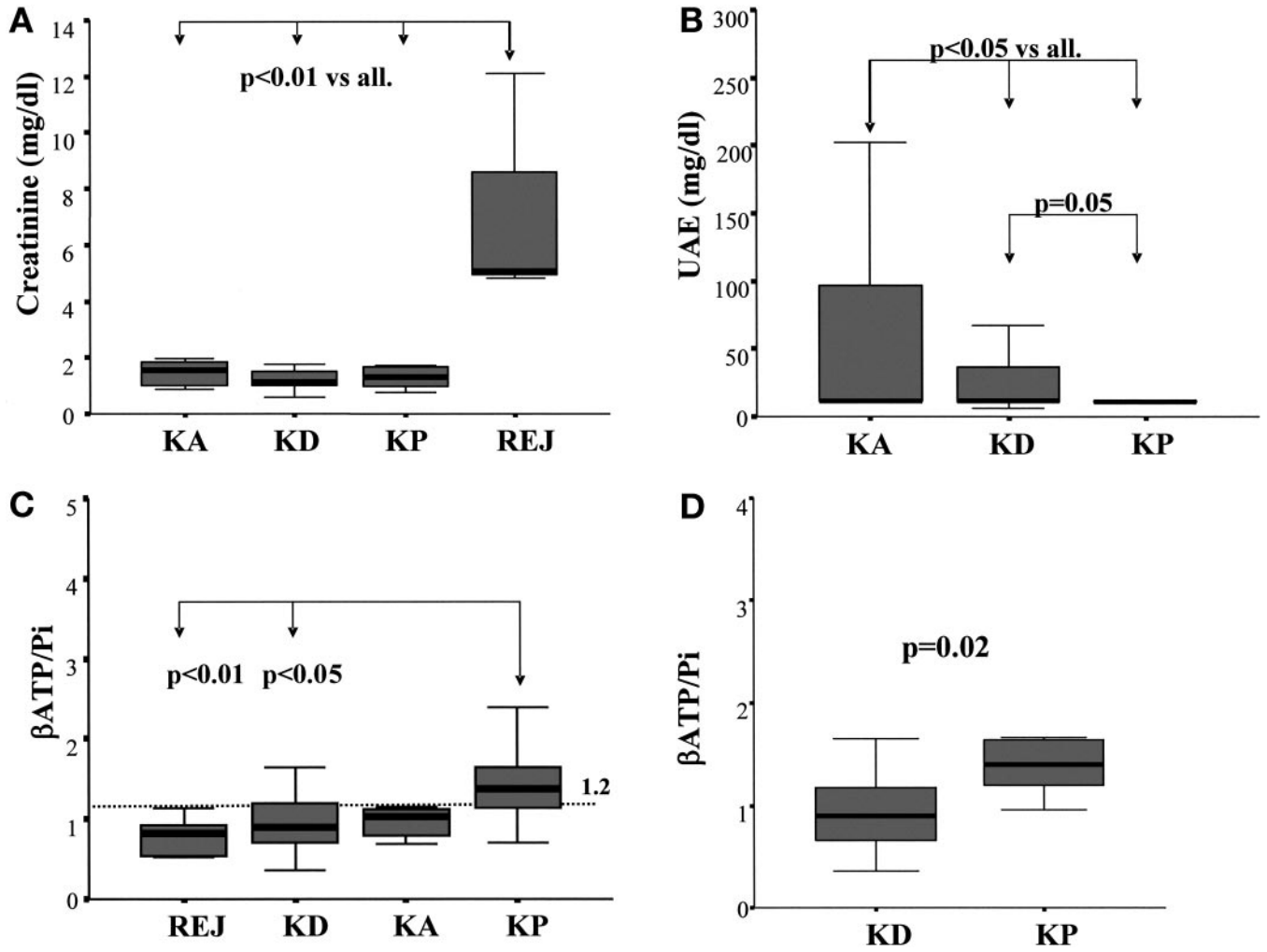

Figure 2—*Similar creatinine values were evident between the groups, except for those who experienced chronic rejection (REJ)* (A). *UAE is higher in the nondiabetic microalbuminuric group (KA) and in the diabetic KD group, compared with the KP transplanted group* (B). *β-ATP/Pi ratio is higher in KP patients compared with KD, KA, or REJ patients* (C). *In the contest of normoalbuminuric patients, KP group showed higher β-ATP/Pi ratio compared with the KD group, suggesting an early alteration of this ratio before the appearance of microalbuminuria* (D). *REJ patients are those with CAN.*

tical package for windows (SPSS, Chicago, IL).

RESULTS

Metabolic and clinical characteristics and kidney function

Cardiovascular risk factors, including age, total cholesterol, HDL, LDL, hypertension, and smoking habits, were similar in the two groups (Table 1). Triglyceride levels were statistically lower in the KP group compared with the KD and REJ groups (Table 1, $P < 0.01$). Patients in the KP group showed, as expected, lower glucose and A1C compared with the KD group (Table 1, $P < 0.001$). Patients in the REJ group, as expected, showed altered lipid metabolism and increased values of arterial blood pressure (Table 1).

No differences between the two groups of transplanted patients were evident with regard to numbers of rejection, HLA match, cold ischemia time, episodes of cytomegalovirus infection, donor age, BMI, and panel reactive antibodies pretransplant, and no infections or lymphoproliferative diseases were evident in the chosen patients (data not shown). Creatinine was <2 mg/dl in all patients in the KP, KD, and microalbuminuric kidney (KA) groups (Fig. 2*A*). UAE rates in all KP and in almost all KD patients were within the physiological range (Fig. 2*B*). However, the KD and KA groups showed higher UAE levels compared with the KP group (Fig. 2*B*). The REJ group exhibited higher creatinine levels than the other three groups (Fig. 2*A*), as well as frank proteinuria in patients who still had the ability to produce urine (data not shown).

β-ATP/Pi ratio in the KP and KD groups

β-ATP/Pi ratio was higher in the KP group compared with the KD group ($P < 0.05$) (Fig. 2*C*). The KP, but not KD, group had a median >1.2, which indicates a probability of graft survival >5 years. We also investigated eventual differences between male and female donors and noticed that KP and KD patients who received a kidney graft from a female donor had a lower β-ATP/Pi ratio. This may be due to the fact that women have a lower density of nephrons and therefore a lower kidney metabolism and kidney β-ATP/Pi ratio.

We also researched a correlation between having a KD or a KP graft with a

level of β-ATP/Pi ratio >1.2. The odds for KD patients having β-ATP/Pi ratio <1.2 are 3, while the odds of having β-ATP/Pi ratio <1.2 for KP patients are 0.5. The REJ and KA groups showed statistically lower β-ATP/Pi ratios than the KP group but not compared with the KD group. Finally, we could not find a correlation between age and β-ATP/Pi ratio (Spearman's ρ −0.13, $P = 0.44$).

Glucose metabolism and β-ATP/Pi ratio

Since nephropathy is the major complication of diabetes, we expect that patients with residual diabetes (the KD group), and therefore high levels of A1C (>6%), will have low levels of β-ATP/Pi ratio and thus will be predisposed for impaired renal function compared with the KP group. We tested whether there is a correlation between levels of A1C and the β-ATP/Pi ratio. Results demonstrate an inverse correlation between A1C levels and β-ATP/Pi ratio with a Spearman's ρ-correlation coefficient of −0.40 and $P = 0.03$ (data not shown). It is important to observe that our KD patients showed a worse glycometabolic control, as shown by high A1C (Table 1). This is not unusual, at least in our clinical experience, and is most likely due to multiple factors such as the use of diabetogenic medications (steroids and calcineurin inhibitors), the presence of a single kidney, and imperfect compliance to insulin therapy. Finally, the early enrollment of KD patients in the islet transplantation waiting list often makes the selection of these patients difficult.

Another important issue is the possible interference between hyperglycemia per se and β-ATP/Pi ratio. In this case, the differences in the spectra could reflect only the differences in degree of diabetes control. We took advantage of a small cohort of patients who received islet transplantation >1 year after the kidney transplant. These patients ($n = 5$) underwent ^{31}P-MRS during a phase of relatively good glycometabolic control (A1C 6.9 ± 0.3%) due to a well-functioning islet transplantation (all patients C-peptide >0.5 ng/ml). In this small group we still detected an impaired β-ATP/Pi ratio (0.87 ± 0.22) but was not statistically different from the KD group despite the different glucose control.

Microalbuminuria and β-ATP/Pi ratio and subanalysis in the normoalbuminuric patients

The KA group, despite the absence of diabetes, showed a lower β-ATP/Pi ratio compared with the KP group, suggesting that β-ATP/Pi ratio is impaired in microalbuminuric patients and is a clear marker of early kidney dysfunction. We assessed the existence of a relationship between UAE and β-ATP/Pi ratio. A negative, although not statistically significant, correlation was observed (data not shown).

It is possible the altered HEP metabolism in the KD group is a consequence of an incipient renal dysfunction, as suggested by higher UAE in this group. We decided to analyze the HEP metabolism in the context of normoalbuminuric patients of the two groups (KP and KD). Even in this smaller cohort, a statistically significant difference was evident in terms of β-ATP/Pi ratio between the two groups (KP 1.78 ± 0.40 vs. KD 0.92 ± 0.12, $P = 0.02$) (Fig. 2*D*).

Lipid metabolism and β-ATP/Pi ratio

It has been shown that augmented lipid and lipoprotein levels increase the risk of graft chronic rejection due to vascular damage. Since cholesterol >190 mg/dl is the major risk factor for atherosclerosis and vascular complications, we investigated the correlation between cholesterol levels and β-ATP/Pi ratio in our cohort of patients. No significant correlation was found with nonparametric Spearman's test (Spearman's $\rho = -0.16$, $P = 0.41$).

Finally, we observed that levels of triglycerides were different among the KP and KD groups ($P < 0.01$ for difference of medians, Table 1). No significant correlation was found with nonparametric Spearman's test (Spearman's $\rho < 0.21$, $P = 0.10$).

Immunosuppressive treatment and β-ATP/Pi ratio

Calcineurin inhibitors have been seen to have a dose-dependent cytotoxic effect on the kidney; they can cause tubular damage and vasospasm of the renal artery (17,18). Alternatively to cyclosporine, FK506 has been used for some patients, although it has a similar pharmacological action and a similar nephrotoxicity (19,20). We therefore hypothesized that high doses of an immunosuppressant could correlate with low β-ATP/Pi ratio. In our samples, results demonstrate no significant correlation (Spearman's $\rho = -0.10$, $P = 0.65$) between doses of cyclosporine and cyclosporine trough levels and β-ATP/Pi ratio (data not shown). The same could be said for FK506, as there is no significant correlation between dose and β-ATP/Pi ratio (data not shown).

Smoking and β-ATP/Pi ratio

We did not detect any influences of smoking on β-ATP/Pi ratio (Spearman's $\rho > 0.30$, $P = 0.55$). Our transplanted patients are aware of the potential negative impact of smoking on their health condition, particularly in terms of cardiovascular problems and cancer progression.

Urinary thromboxane B2/6-keto-prostaglandin-F-1α levels and β-ATP/Pi ratio

We evaluated urinary thromboxane B2 and 6-keto-prostaglandin-F-1α levels in KP and KD groups as markers of early deterioration of glomerular function (16). It is particuarly known that in early stages of diabetes, an elevation of glomerular filtration rate is evident, with an increase in urinary prostaglandins (16). These two markers appeared to be augmented during hyperfiltration and during the early stages of diabetes or kidney disease. The KP group showed nonstatistical lower levels of urinary 6-keto-prostaglandin-F-1α and urinary thromboxane B2 (data not shown). Despite the small patient cohort, a negative trend was evident between urinary thromboxane B2 and 6-keto-prostaglandin-F-1α levels and β-ATP/Pi ratio (data not shown).

Sensitivity and specificity of kidney spectroscopy

An estimate of intra-examination differences in β-ATP/Pi was obtained in seven transplanted subjects in which the spectroscopy scan was obtained twice in a consecutive fashion on the same session and without changing the position of the surface coil, the sensitive volumes, and the acquisition parameters. The coefficient of variation was 6 ± 3%. Due to the considerably smaller signal-to-noise ratio, the variability was higher in the nondiabetic kidney transplanted patients with overt CAN (group REJ) (15 ± 5% tested in three patients).

CONCLUSIONS — Chronic allograft dysfunction is one of the major problems that transplanted patients face and still represents one of the most important causes of graft loss (2,5,21,22). Many fac-

tors can detrimentally accelerate the burden of CAN including hypertension, dyslipidemia, overactive kidney, kidney cold ischemia, and nephrotoxicity of certain drugs (5,23–27). It is particularly well known that both calcineurin inhibitors and rapamycin are nephrotoxic (5,23–27), as they can induce tubular injury and glomerular vasoconstriction, leading to the worsening of kidney function (5,23–27). Most patients who experience CAN restart dialysis and ultimately need retransplantation. The presence of diabetes is, of course, another worsening factor. We and others showed that restoring β-cell function, with either islet or pancreas transplantation, can improve graft survival (12,13,28). From a clinical point of view, once the injury is established, it is unlikely that the pathological loop can be overturned; it is important to discover more noninvasive tools to use at the beginning of CAN in order to determine a propensity to develop chronic allograft dysfunction.

Microalbuminuria, while it is an early and noninvasive diagnostic tool, is also sometimes nonspecific and difficult to treat. In recent years, imaging techniques showed great potential in studying metabolic and vascular features of kidney grafts (6,8,10,29,30). We and others recently published (13,29) the use of Doppler imaging to evaluate intrarenal blood flow, which joined kidney biopsy as a tool to detect abnormalities in kidney vessel morphology and functionality. MRS in one of the most up-to-date tools to noninvasively study organ metabolism (6,8,10,29–31). In this work, we aimed to use localized ^{31}P-MRS to detect early signs of kidney graft dysfunction before the appearance of microalbuminuria and to detect a possible early beneficial effect of combined KP transplantation on kidney graft metabolism.

In our study, ^{31}P-MRS showed that combined KP transplantation, compared with KD transplantation, is associated with a better HEP metabolism in the kidney graft, using a cross-sectional approach. β-ATP/Pi ratio appeared to be correlated with A1C, suggesting a significant effect of glycometabolic control on HEP metabolism. Finally, these data were confirmed even in normoalbuminuric patients when there are no evidences of kidney dysfunction.

Pancreas cotransplantation can halt the progression of diabetic nephropathy in KP patients ameliorating HEP metabolism, thereby improving the survival of the kidney graft, ameliorating vascular function, and preventing the reduction of renal functional reserve (4,11,32). ^{31}P-MRS can be helpful for detecting early dysfunction of the kidney graft long before the appearance of an overt disease. We would like to call attention to the fact that among the measured parameters, an impairment of β-ATP/Pi ratio is the first to appear even in normoalbuminuric patients. While creatinine levels and albumin excretion rate are normal in all patients, β-ATP/Pi ratio is lower in patients with type 1 diabetes after KD transplantation than in patients with type 1 diabetes after KP transplantation.

The utility of β-ATP/Pi ratio as a marker of renal dysfunction is evidenced by comparing control groups with different degrees of kidney dysfunction (33). From normoalbuminuric (KP group) to microalbuminuric (KA or, to a lesser extent, KD group) to frank proteinuric (REJ group) patients, a progressive worsening of β-ATP/Pi ratio is evident. We analyzed early markers of renal hemodynamics such as urinary thromboxane B2 and 6-keto-prostaglandin-F-1α. Both are increased in type 1 diabetic patients and in other kidney diseases during the early hyperfiltration phase. KD patients showed increased levels of both urinary markers, suggesting that they are hyperfiltrating. Interestingly, in diabetic patients, β-ATP/Pi ratio showed a negative correlation, though not statistically significant, with urinary thromboxane B2 and 6-keto-prostaglandin-F-1α.

Glucose control does not seem to alter, at least acutely, HEP metabolism, as shown by the analysis of kidney-islet transplanted patients. Despite lower glycemia, they still showed altered HEP metabolism.

Limitations of the study

The major limitation of this study is the use of a cross-sectional study, although the two populations were homogeneous before transplantation. β-ATP/Pi ratio of kidney graft was not specifically studied at the moment of transplant, and we cannot completely exclude the presence of some unstudied biases. Furthermore, we acknowledge the complexity of the procedure and the lack of extensive studies addressing the specificity and sensibility of the procedures.

Our study shows a higher ratio of β-ATP/Pi in ESRD type 1 diabetic patients who underwent KP transplant compared with those who underwent KD transplant. This could have direct consequences on the prognosis of the graft if we believe that an altered HEP metabolism is the sign of early kidney graft dysfunction. These data confirm that KP transplantation is protective (compared with KD transplantation) for chronic rejection or for recurrence of diabetic nephropathy as previously suggested by others (12,13,28,34). Furthermore, we demonstrated for the first time that even in the absence of an overt renal dysfunction (as suggested, for example, by increased creatinine levels or by the appearance of proteinuria), the kidney graft showed early markers of impairment that could be used as a target for therapy. Our data confirmed the body of evidence that restoration of β-cell function is helpful for the kidney graft with important metabolic effects. The importance of our findings has to be validated with longitudinal studies addressing particularly the sensibility of ^{31}P-MRS in predicting graft dysfunction. Only such an approach will confirm the real value of ^{31}P-MRS as a useful clinical technique.

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