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# AN ANALYSIS OF PRETRANSPLANTATION VARIABLES ASSOCIATED WITH LONG-TERM ALLOGRAFT OUTCOME IN PEDIATRIC LIVER TRANSPLANT RECIPIENTS RECEIVING PRIMARY TACROLIMUS (FK506) THERAPY

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***Background.*** **The present study analyzes pretransplantation variables associated with long-term liver allograft survival in 278 children who underwent transplantation under primary tacrolimus (FK506) therapy at a single center between October 1989 and October 1996.**

***Methods.*** **The influence of 17 pretransplantation variables on long-term liver allograft outcome was analyzed. Donor variables included age, weight, gender, and cold ischemia time. Recipient variables included age, weight, gender, original liver disease, pretransplantation waiting time, previous abdominal surgery, United Network of Organ Sharing (UNOS) status, ABO blood group, bilirubin level, prothrombin time, ammonia level, creatinine level, and reduced-size/split liver grafts.**

***Results.*** **Overall actuarial graft survival was 79.9% at 1 year, 79.1% at 2 years, and 78.3% at 3, 4, and 5 years. Retransplantation rate was 10.8%. Pretransplantation variables with a significant adverse effect on graft survival by univariate analysis were donor age ≤1 year ($P<0.004$), donor weight ≤10 kg ($P<0.003$), UNOS status I and II ($P<0.007$), ABO type O, B, and AB ($P<0.03$), and reduced-size/split liver grafts ($P<0.02$).**

**Pretransplantation variables significant by multivariate analysis and therefore independent predictors of inferior graft outcome were donor weight ≤10 kg (relative risk [RR] 2.91, confidence interval [CI] 1.53–5.51); reduced-size/split liver grafts (RR 2.53, CI 1.30–5.64); and UNOS status I (RR 2.22, CI 1.11–4.43).**

***Conclusions.*** **Pediatric liver transplant recipients receiving primary tacrolimus therapy have long-term graft survival rates approaching 80%. UNOS status, donor weight, and the use of reduced-size/split liver grafts are the most important factors affecting survival.**

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Orthotopic liver transplantation (OLTx*) in children is presently being performed with graft survival rates ranging from 68% to 88% at 1 year and 55% to 68% at 5 years after transplantation (*1*). Refinements in surgical techniques, better immunosuppressive agents, and improved management of infectious complications have all contributed to an improved survival rate compared with an early report from the Children's Hospital of Pittsburgh (*2*). Although several reports have been published analyzing pretransplantation risk factors and liver allograft outcome in the adult population (*3–6*), fewer reports exist that deal exclusively with predictors of graft survival in pediatric patients (*7–9*).

The use of tacrolimus (FK506) induction therapy in pediatric OLTx has met with encouraging results as noted in recent reports (*10, 11*). Since 1989, all pediatric OLTx recipients at Children's Hospital of Pittsburgh have received tacrolimus induction therapy for first liver allografts. The present study analyzes the influence of pretransplantation variables on long-term primary liver allograft outcome in these children to determine predictors of graft success or failure.

## PATIENTS AND METHODS

From October 1989 through October 1996, 278 pediatric patients received 310 liver allografts at the Children's Hospital of Pittsburgh. Only data and results for the 278 primary liver allografts were included in this study. Information was retrospectively collected from the databases at the Thomas E. Starzl Transplantation Institute and Children's Hospital of Pittsburgh. Recipient selection was based on medical need, liver size, and ABO compatibility. Mean age of all patients was 4.9±0.3 years (median 2.6, range 0.07–17). Mean follow-up was 55.9±1.9 months (median 60.0, range 0.0–98.9). Candidates for OLT were assigned to one of the following United Network for Organ Sharing (UNOS) categories depending on medical condition: status 1, in intensive care unit (ICU) with expected survival less than 7 days; status 2, continuously hospitalized; status 3, at home but requiring continuous medical care; and status 4, at home and relatively functional.

Liver procurement and graft implantation was performed by techniques previously described (*12, 13*). All grafts were flushed with the University of Wisconsin solution. Decisions to perform liver reductions or split liver transplantation depended on the size of the liver, the size of the abdominal cavity of the recipient, and medical urgency. Between October 1989 and July 1996, split liver transplantation was performed by an ex vivo technique, whereas in situ splitting in a heart-beating cadaveric donor was initiated in August 1996.

All OLTx recipients were treated with primary tacrolimus (Prograf, Fujisawa USA, Inc., Deerfield, IL) induction therapy (0.05–0.075 mg/kg/12 hr intravenously) with conversion to oral therapy when tolerated. Initial corticosteroid therapy consisted of 10 mg/kg bolus of intravenous methylprednisolone in the operating room followed by a corticosteroid taper of 5 mg/kg at postoperative day 1 to 1 mg/kg at postoperative day 5 with a 20%/week reduction thereafter. Maintenance prednisone therapy was individualized with attempts to wean completely off steroids when possible. No patients received antilymphocyte/thymocyte preparations, azathioprine, or mycophenolate mofetil as induction therapy.

The influence of 17 pretransplantation variables on long-term primary liver allograft outcome was analyzed in all children. Donor variables included age (≤ or >1 year), weight (≤ or >10 kg), gender (male/female), and cold ischemia time. Recipient variables analyzed were age (≤ or >2 years), weight (≤ or >10 kg), gender (male/female), etiology of liver disease, presence or absence of previous abdominal surgery, UNOS status (I, II, or III), ABO blood group (O, A, B, or AB), full-sized versus reduced-size/split liver grafts, pretransplantation bilirubin level, prothrombin time, ammonia level, and creatinine level. Overall actuarial first graft survival was calculated and causes of death and graft loss were examined.

*Statistical analysis.* Univariate analysis was performed initially on all pretransplantation variables. The Kaplan-Meier method with a log-rank test was used to find predictors of graft failure. For that purpose, continuous variables were presented as categorical based on clinically established cut-off points (i.e., donor weight, donor age, recipient weight, and recipient age). $P$ values less than 0.05 were considered significant. All continuous variables are reported as mean ± SEM with categorical variables reported as proportions.

Variables found with univariate analysis to be associated with outcome were then entered into a stepwise backward Cox proportional hazard model to determine variables that are independent predictors of outcome. All the included variables met the assumption of the proportional hazard. Variables were considered eligible for removal from the model if the likelihood ratio test significance was greater than or equal to 0.1. Based on the results of multivariate analysis, subsequent subgroup case analysis was performed.

Statistical analysis was performed using the SPSS/PC+ Advanced Statistics Package, Version 8.0 (Cary, NC).

## RESULTS

Overall actuarial patient survival was as follows: 86.7% at 1 year, 85.9% at 2 years, 85.2% at 3 years, and 84.7% at 4 and 5 years. Actuarial graft survival was 79.9% at 1 year, 79.1% at 2 years, and 78.3% at 3, 4, and 5 years.

*Univariate analysis. Donor risk factors:* The impact of donor age and donor weight on graft survival is shown in Table 1. Inferior graft survival was noted in children receiving liver allografts from donors less than 1 year of age ($P<0.004$ vs. donors >1 year of age) and less than 10 kg in weight

TABLE 1. Donor variables according to graft outcome

| | Successful grafts (n=218) | Failed grafts (n=60) | $P$ value |
|---|---|---|---|
| Age[a] | | | $P<0.004$ |
| ≤1 year | 18% (40) | 35% (21) | |
| >1 year | 82% (177) | 65% (39) | |
| Weight[a] | | | $P<0.003$ |
| ≤10 kg | 22% (48) | 41% (24) | |
| >10 kg | 78% (166) | 59% (34) | |
| Gender (M/F) | 126/92 | 39/21 | $P$=NS |
| Cold ischemia time (hr) | 12.1±0.3 | 12.2±0.5 | $P$=NS |

[a] The difference between total number of patients for each variable and 278 is due to the missing values for a particular variable. Percent was calculated for available values only.

* Abbreviations: ICU, intensive care unit; OLTx, orthotopic liver transplantation; PNF, primary nonfunction; PTLD, posttransplant lymphoproliferative disorder; UNOS, United Network for Organ Sharing.

($P<0.003$ vs. donors >10 kg). Donor gender and cold ischemia time did not influence allograft outcome.

*Recipient risk factors:* The impact of recipient pretransplantation variables on allograft outcome are shown in Table 2. Variables adversely affecting allograft survival included UNOS status ($P<0.007$, I vs. II vs. III), ABO blood group ($P<0.03$, O, B, AB vs. A), and the use of reduced-size/split liver grafts ($P<0.02$). A trend toward inferior graft survival was noted in recipients less than 2 years of age ($P<0.16$ vs. recipients >2 years) and less than 10 kg in weight ($P<0.07$ vs. recipients >10 kg).

Analysis of the other remaining recipient pretransplantation variables revealed no association with graft outcome. No differences in values were seen between successful and failed grafts when total bilirubin levels (12.1±0.8 vs. 14.9±1.6 mg/dl, $P$=NS), prothrombin time (15.8±0.4 vs. 16.4±0.6 seconds, $P$=NS), serum ammonia levels (73.8±3.9 vs. 82.7±9.9 μmol/L, $P$=NS), and serum creatinine levels (0.45±0.06 vs. 0.46±0.09 mg/dl, $P$=NS) were examined.

*Multivariate analysis. Graft losses in study period:* Three variables were shown to be independent predictors of graft failure and are depicted in Table 3 along with the relative risk of graft loss. These independent predictors of graft loss were donor weight less than 10 kg, the use of reduced-size/split liver grafts, and recipient UNOS status I. The calculated probability of graft failure was 2.91 times greater if a donor weighed ≤10 kg; 2.53 times greater if a reduced-size/split liver was used; and 2.22 times greater if the recipient was UNOS status I. Graft survivals for the three high-risk pretransplantation variables are shown in Figure 1, A–C.

TABLE 2. Recipient pretransplantation variables according to graft outcome

| | Successful grafts (n=218) | Failed grafts (n=60) | P value |
|---|---|---|---|
| Age | | | $P<0.16$ |
| ≤2 years | 43% (94) | 53% (32) | |
| >2 years | 57% (124) | 47% (28) | |
| Weight[a] | | | $P<0.07$ |
| ≤10 kg | 41% (77) | 56% (29) | |
| >10 kg | 59% (109) | 44% (23) | |
| Gender (M/F) | 125/93 | 34/26 | $P$=NS |
| Original liver disease (%) | | | $P$=NS |
| Cholestatic/biliary atresia | 56% (122) | 50% (30) | |
| Metabolic | 16% (36) | 12% (7) | |
| Fulminant hepatic failure | 10% (21) | 12% (7) | |
| Miscellaneous | 18% (39) | 26% (16) | |
| Pretransplantation waiting time (mo) | 6.5±1.3 | 3.2±0.6 | $P$=NS |
| Previous abdominal surgery (%) | 52% (113) | 58% (35) | $P$=NS |
| UNOS status (%) | | | $P<0.007$ |
| 1 | 28% (62) | 50% (30) | |
| 2 | 24% (52) | 20% (12) | |
| 3 | 48% (104) | 30% (18) | |
| ABO blood group (%) | | | $P<0.03$ |
| O | 42% (91) | 47% (28) | |
| A | 41% (90) | 23% (14) | |
| B | 12% (26) | 23% (14) | |
| AB | 5% (11) | 7% (4) | |
| Liver size | | | $P<0.02$ |
| Reduced-size/split liver | 8% (17) | 17% (10) | |
| Full-size liver | 92% (201) | 83% (50) | |

[a] The difference between total number of patients for each variable and 278 is due to the missing values for a particular variable. Percent was calculated for available values only.

TABLE 3. Independent predictors of inferior graft outcome in pediatric orthotopic liver transplant recipiets

| | Relative risk | 95% Confidence interval | P Value |
|---|---|---|---|
| Donor weight ≤10 kg | 2.91 | 1.53–5.51 | $P<0.001$ |
| Reduced-size/split liver | 2.53 | 1.30–5.64 | $P<0.02$ |
| UNOS status I | 2.22 | 1.11–4.43 | $P<0.05$ |

*Graft losses in recipients with a combination of high-risk pretransplantation variables:* To insure clinical applicability of the results of the multivariate model, the following analysis was performed. Based on independent predictors of graft survival, recipients were stratified according to the presence (donor weight ≤10 kg or reduced-size/split liver graft and UNOS status I, n=20) or absence (donor weight >10 kg, full-sized liver, and UNOS status II or III, n=122) of high-risk pretransplantation variables, and graft survival was calculated by Kaplan-Meier statistics (Fig. 1D). Recipients with a combination of pretransplantation risk factors had significantly lower graft survival compared with recipients without risk factors (50.0% and 45.0% vs. 89% and 89% at 1, and at 2, 3, and 5 years, respectively, $P<0.00001$). Mean survival time was 45.2±10.5 months in the high-risk group and 88.7±2.7 months in the low-risk group.

*Overall causes of liver allograft loss.* A total of 60 (21.6%) primary grafts were lost, with 28 (10%) functioning grafts lost as a result of patient deaths. The causes of graft loss were as follows: 17 (53.1%), vascular thrombosis; 12 (37.5%), primary nonfunction (PNF); 1 (3.1%), adenovirus hepatitis; 1, disseminated arteritis; and 1, chronic rejection. Causes of graft loss resulting from patient deaths were sepsis, 13 (46.4%); posttransplant lymphoproliferative disorder (PTLD), 5 (17.9%); neurologic, 4 (14.3%); subclavian artery laceration, 1 (3.6%); graft-versus-host disease, 1; metastatic hemangiosarcoma, 1; recurrent giant cell hepatitis, 1; and ruptured splenic artery and hepatic artery aneurysms, 1 each.

*Causes of graft loss in recipients with high-risk variables. Donor weight less than 10 kg:* Twenty-four of 72 (33.3%) grafts were lost in this group. Graft loss attributed to graft-related problems occurred in 16 (66.7%) patients: 12 (50%) vascular thrombosis and 4 (16.7%) PNF. Eight patients (33%) died with functioning grafts as follows: sepsis, 4; PTLD, 2; subclavian artery laceration, 1; and neurologic, 1.

*Reduced-size/split liver grafts:* Of 27 partial liver grafts performed, 14 were reduced-size and 13 were split liver grafts (11 ex vivo and 2 in situ). Four of 14 (28.6%) reduced-size grafts were lost: 2, PNF; 1, hepatic artery thrombosis; and 1, chronic rejection. In the split liver group, 6 of 13 (46.1%) grafts were lost (all performed by the ex vivo technique): 3, PNF; 2, hepatic artery thrombosis; and 1 neurologic complication resulted in a patient death.

*UNOS status I:* A total of 92 UNOS status I patients who underwent transplantation with 30 (32.6%) graft losses. Causes of graft loss in this group were vascular thrombosis, 11 (36.7%); PNF, 3 (10%); and chronic rejection, 1 (3.3%). Death as a cause of graft loss occurred in the remaining 15 (50%) patients as follows: sepsis, 6; neurologic, 4; PTLD, 3; recurrent hemangiosarcoma, 1; and subclavian artery laceration, 1.



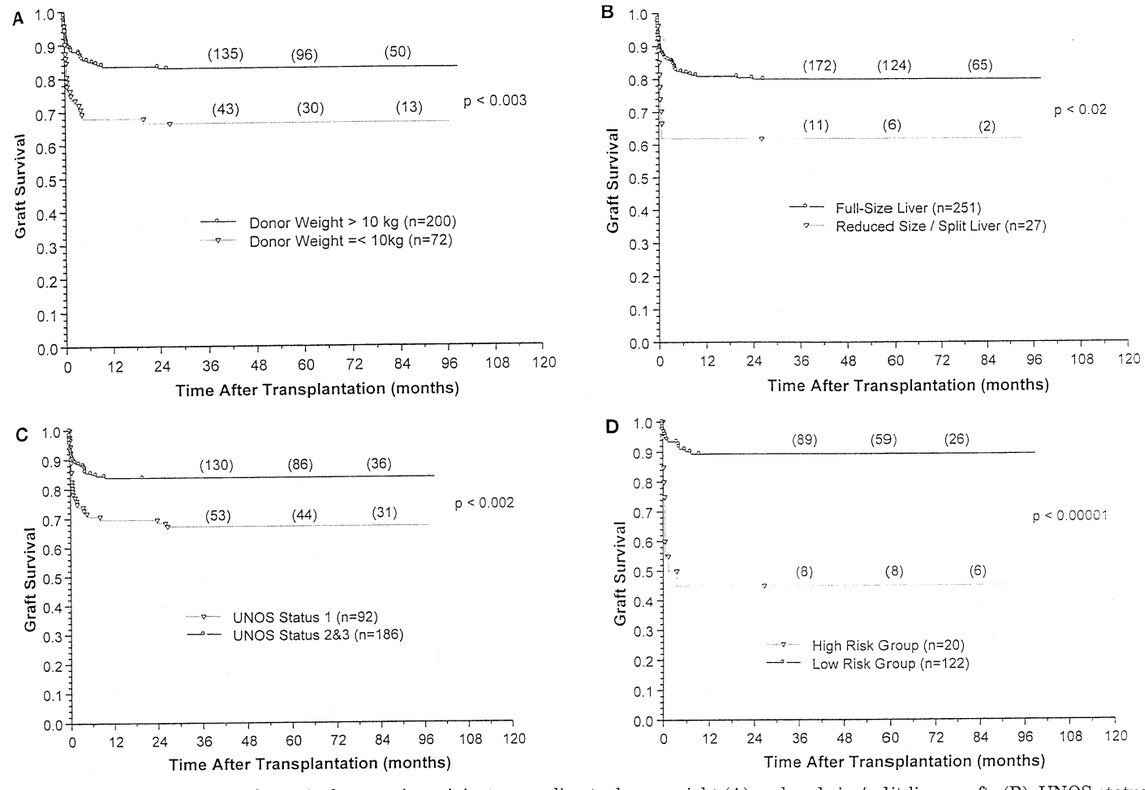


FIGURE 1. Kaplan-Meier graft survival curves in recipients according to donor weight (A), reduced-size/split liver grafts (B), UNOS status (C), and the presence or absence of a combination of high-risk pretransplantation variables (D).

## DISCUSSION

Pediatric OLTx has undergone many advancements since the first attempts by Starzl in the 1960s such that liver replacement is now the main treatment for children with end-stage liver disease. The introduction of tacrolimus into clinical OLTx and the improved results reported, compared with cyclosporine-based immunosuppression, have been contributing factors (*10*, *14*, *15*). With the increasing success of OLTx in children, as well as adults, several studies have attempted to identify risk factors associated with patient and graft survival in OLTx (*7–9*). The present report focuses specifically on pretransplantation variables and their impact on long-term graft outcome in pediatric OLTx recipients receiving tacrolimus induction therapy. In addition, this study also allows an opportunity to examine long-term graft survival and causes of graft loss in tacrolimus recipients.

The patient and graft survival rates with tacrolimus in the present study compare favorably to other large series of pediatric OLTx recipients (*9*, *16*), although these other series all used varying immunosuppressive regimens. The survival statistics presented in our series are noteworthy in that all patients were consecutively treated with tacrolimus with no exclusionary criteria, a uniform immunosuppressive regimen was used, and posttransplantation follow-up was relatively long. Therefore, it seems that tacrolimus can be used safely in pediatric OLTx recipients with good patient and graft survival over time.

When causes of graft loss were examined, approximately one half were due to loss of the graft itself while the remaining grafts were lost due to patient deaths. Of particular interest, only one graft was lost to chronic rejection, whereas 48% of graft losses were due to technical problems or PNF and 22% to septic complications. This low rate of graft loss from immunologic complications (1/278, 0.36%) is similar to other centers that have noted, like our study, a large percentage of grafts loss secondary to technical and infectious factors (*17*, *18*).

Previous studies have noted the influence of donor variables on outcome in pediatric OLTx recipients (*8*, *9*). Because of the small body size of many pediatric donors and recipients, technical complications, especially vascular thromboses, have been well described (*19*, *20*). These findings are in agreement with data from the present study, which showed low donor weight and young donor age to adversely effect graft survival. When causes of graft loss were examined in the group of donors ≤10 kg, 50% were due to vascular thrombosis. The association of low donor weight, subsequent vascular thrombosis and decreased graft survival is similar to a report that showed a 25% decrease in survival in infants with

hepatic artery thrombosis (*21*). Attempts to reduce the incidence of graft loss due to technical problems in this donor group have been undertaken with the routine use of dextran, intravenous heparin, and aspirin in the early postoperative period.

According to UNOS data, 8–10% of children less than 5 years of age die on the waiting list while awaiting OLTx (*1*). This high waiting list mortality is due to the discrepancy between the number of small pediatric patients awaiting OLTx and the number of available pediatric donors. Attempts to alleviate this problem have led to innovative techniques, including reduced-size, split liver, and living-related OLTx, to increase the donor pool size for infants and small children (*22–25*). No difference in survival has been shown between recipients of reduced-size liver allografts and full-sized grafts (*22, 23*); early experience with split liver transplantation has shown inferior graft survival in recipients of split liver grafts (*24*), however, more recent results have been encouraging (*26, 27*). The present study has shown the use of reduced-size/split liver grafts to be a risk factor for graft loss. Examination of graft losses in this group of patients reveals the majority of losses (8/10) to be related directly to the graft itself (three vascular thromboses and five PNF). In fact, the majority of graft losses occurred in the ex vivo split liver group, a technique we have since abandoned in favor if in situ splitting, with the expectation that a learning curve will be present during an initial in situ split liver experience. With one center recently reporting patient and graft survival rates of 92% and 86%, respectively, with in situ split liver transplantation (*28*), it seems this technique is presently a viable alternative for increasing the donor pool for small children.

Several recipient variables were noted to adversely impact on graft survival. Recipient condition at time of OLTx, as determined by UNOS status, was associated with degraded graft survival. Children in more dire circumstances at the time of OLTx, that is those hospitalized in an ICU setting, fared more poorly than those who underwent transplantation while waiting at home. This was significant only in children with chronic liver failure who developed life-threatening complications because, interestingly, when the effect of fulminant hepatic failure on graft survival was examined, no difference was noted between it and other causes of liver disease. Time on the waiting list and progression of disease as measured by biochemical parameters (bilirubin, prothrombin time, creatinine level, and ammonia level), however, exerted no influence on graft outcome in contrast to other reports (*22, 29*). It should be mentioned that several variables that measure disease progression that were not examined in the present analysis (such as encephalopathy, malnutrition, and ascites accumulation) have been previously identified as risk factors (*6, 29*).

UNOS status and its impact on graft outcome has been previously described in several studies with UNOS status I recipients representing a higher risk group (*1, 8, 16*). When causes of graft loss in this group of patients were identified, 20% were due to septic complications, a not unexpected outcome in ICU-bound patients. The remaining causes of graft loss, however, were varied with almost 50% of graft losses due to graft-related problems (vascular thrombosis and PNF). It therefore seems that causes of graft loss in these ICU-bound patients varied with patient, graft, and technical factors all contributing to decreased graft survival. Despite the poorer outcome in UNOS status I recipients, we believe these patients should still be given highest priority for OLTx and subsequently undergo transplantation in a timely fashion.

The present study has identified several risk factors that adversely effect graft survival, namely low donor weight, hospitalized ICU patients, and the use of reduced-size/split liver allografts. Because of the shortage of suitable donors for pediatric patients, and organ donor shortage in general, the avoidance of using low weight donors is impractical. Likewise, transplantation of hospitalized patients will always be a reality in liver transplantation. The use of split liver transplantation is a modality that will likely increase in the future. By continuing technical refinements and expanding the donor pool, perhaps improvements in graft survival can be made such that low weight donors, split liver transplantation (especially by the in situ technique), and advanced UNOS status are no longer risk factors.

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# EFFECTS OF CRYOPRESERVATION ON IN VITRO AND IN VIVO LONG-TERM FUNCTION OF HUMAN ISLETS[1]

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***Background.*** **The possibility of performing transplantation several days after explant seems to be a peculiarity of islet grafts, and the opportunity to cryopreserve human islets may permit an indefinite period for modulating the recipient immune system. The aim of the present study was the evaluation of in vitro and in vivo functional properties of cryopreserved human islets.**

***Methods.*** **We used six consecutive human islet preparations not suitable for an immediate transplantation in diabetic patients because the limited islet mass separated. The in vitro function of cryo and fresh islets was studied by determination of insulin and glucagon secretion in response to such classical stimuli as glucose (16.7 mM), glucose (16.7 mM) + 3-isobutyl-1-methylxanthine (0.1 mM), arginine (10 mM), and tolbutamide (100 μM). In vivo islet function was assessed through intravenous glucose tolerance tests performed at 15, 30, 60, and 90 days after transplantation of 1000 hand-picked fresh or cryopreserved islets in nude mice.**

***Results.*** **Basal secretion of true insulin was significantly higher in cryopreserved islets than in fresh ones. The response of cryopreserved islets to arginine and glucose + isobutyl-1-methylxanthine seemed partially impaired. Proinsulin-like molecule secretion seemed higher in cryopreserved than in fresh islets in response to all secretagogues used, and the difference was statistically significant for arginine. The capacity of human cryopreserved islets to maintain a correct metabolic control in diabetic nude mice was progressively lost in 3 months.**

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