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Islet of Langerhans isolation from pediatric and juvenile donor pancreases

Raphael P. H. Meier,[1]* Ismail Sert,[1,2]* Philippe Morel,[1] Yannick D. Muller,[1] Sophie Borot,[1,3] Lionel Badet,[4] Christian Toso,[1] Domenico Bosco[1] and Thierry Berney[1]

1 Department of Surgery, Cell Isolation and Transplantation Center, University of Geneva Hospitals and School of Medicine, Geneva, Switzerland
2 Department of Organ Transplantation, Tepecik Training and Research Hospital, Izmir, Turkey
3 University of Franche-Comté, Besançon, France
4 Department of Urology and Transplantation, University of Lyon, Lyon, France

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Correspondence
Thierry Berney MD, MSc, Visceral and Transplant Surgery, University of Geneva Hospitals and Medical School, Rue Gabrielle-Perret-Gentil 4, 1211 Genève 14, Switzerland.
Tel.: +41 22 372 34 04;
fax: +41 22 372 76 89;
e-mail: thierry.berney@hcuge.ch

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*RPHM and IS contributed equally to the article and both hold first authorship.

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Summary

Islet grafts isolated from young donors allow superior functional outcomes but are often associated with poor islet isolation yields. The objective of this study was to comparatively analyze the outcomes of islet isolation between young and older donors. We retrospectively analyzed 564 pancreas isolations performed at our institution. Isolation outcomes were compared between donors aged ≤20 years (n = 42, YD) and >20 years (n = 522, OD). Isolation procedure was identical in both groups. Prepurification percentage of embedded islets was higher in YD (44.3 ± 22.7% vs. 24.9 ± 20.9%, $P < 0.001$). This led to a lower recovery rate in YD (48% vs. 76%, $P = 0.002$) and hence lower postpurification IEQ/g pancreas in YD (2 412 ± 1 789 IEQ/g vs. 3 194 ± 1 892 IEQ/g, $P = 0.01$). Final yield was 180 982 ± 128 073 IEQ in YD and 244 167 ± 134 137 IEQ in OD, ($P = 0.006$). *In vitro* function was markedly, albeit nonsignificantly, higher in YD (SI: 4.5 ± 5.1 vs. 3.0 ± 5.7, $P = 0.350$). Proportion of transplanted preparations was similar in both groups, 38% (16/42) in YD vs. 43% (224/522) in OD, $P = 0.628$. In spite of isolation and purification difficulties, pancreases from young donors allowed similar islet transplantation rates as older donors. Efforts should be directed at improving islet extraction in these donors to realize their full potential for islet transplantation.

Introduction

Since the introduction of the Edmonton Protocol, pancreas islet transplantation has become an attractive treatment modality for patients with type 1 diabetes mellitus [1,2], and results have consistently improved in the past decade [3]. Donor age is an important determinant of the outcome of the islet isolation procedure. Transplanted islet preparations from younger donors (YD) are associated with better clinical outcomes [4]. Conversely, some studies have identified that YD are associated with low islet yields [5–7]. This is mainly because of the difficulty of efficiently separating the islets from the surrounding exocrine tissue. Accordingly, many islet isolation centers are likely to turn down pancreas offers from donors aged <20 years [8–10]. However, this attitude is being challenged because of the superior function of islets from young donors [4,8] and the possibility to adapt isolation protocols to the juvenile donor situation to improve islet yields [9].

The aim of this study was to review the outcomes of islet isolation procedures performed at our institution using YD pancreases (≤20 years) and to compare the results with those of pancreases from older donors (OD; >20 years).

Material and methods

Donors

The results of 596 consecutive human islet isolations performed at our institution between 2002 and 2013 were prospectively entered into a database and retrospectively reviewed and analyzed. Thirty-two isolations were excluded from the analysis. The reasons for exclusion were as follows: autotransplantation ($n = 17$) [11], technical failure leading to early interruption of the isolation procedure (YD: $n = 1$; OD: $n = 5$), early interruption of the isolation process before the purification process owing to an initial islet count <50 000 islet equivalent (IEQ) ($n = 5$, all OD), discovery of a tumor (cystadenoma) leading to early interruption of the isolation procedure ($n = 1$), and missing isolation procedure data ($n = 3$). Pancreases were retrieved from brain dead donors from Swiss and French centers participating in the GRAGIL cooperative project [12–14]. Acceptance criteria were identical for younger and older donors and included: cardiac arrest ≤10 min with cardiopulmonary resuscitation performed by health care professionals; serum lipase, AST, and ALT <3× upper limit of normal; negative HIV, HBV (HBsAg, HBcAb), and HCV serologies; intensive care unit stay ≤7 days; cold ischemia time <12 h; and secondary warm ischemia time <120 min. Pancreases were then shipped to the islet isolation facility for processing, in cold (4 °C) preservation solution.

Islet isolation

Isolations were performed as previously described, according to a local adaptation of Ricordi's semi-automated technique [15,16]. Four types of collagenase were used for pancreatic digestion: Collagenase NB1 and NB2 (Serva Electrophoresis, Heidelberg, Germany), Liberase HI (Roche, Indianapolis, IN), and VitaCyte (VitaCyte LLC, Indianapolis, IN). Pancreases were weighed before starting the isolation process, and pancreatic tissue remnants were weighed at the end of the digestion phase. Digestion rate was defined as 100 × (pancreas weight – remnant weight)/pancreas weight (%). Islets were purified on a continuous Biocoll gradient (Biochrom, Berlin, Germany) [16] using a refrigerated COBE cell processor (COBE 2991; Cobe, Lakewood, CO).

Islet quantity and quality assessment

Islet counting and purity assessment were performed before and after purification as previously described [17]. The number of islet equivalents (IEQ) was calculated by normalizing the islets to a standard diameter of 150 μm [18]. Mean pre- and postpurification islet size (μm) was calculated as follows: 150 × IEQ/islet number. The recovery rate (%) was defined as the ratio of postpurification IEQ to prepurification IEQ. A final yield ≥250 000 IEQ was defined as a successful isolation. Transplant volume, viability, purity, endotoxin levels, and functionality of islet preparations were assessed just before transplantation ($n = 240$). Islet viability was assessed by fluorescein diacetate and propidium iodide staining as previously described [19]. Endotoxin levels were measured using the Endosafe-Portable Test System (Charles River Laboratories, Wilmington, MA). *In vitro* function was assessed using a static glucose-stimulated insulin release assay. Stimulation index was calculated as the ratio of insulin concentration of stimulated (high glucose, 16.7 mM) to basal (low glucose, 2.8 mM) conditions.

Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD). Categorical variables are presented as frequency (%). Statistical analysis was performed using the IBM SPSS 21 software (IBM SPSS, Chicago, IL). Unpaired Student's *t*-test ($n > 20$ per group) or Mann–Whitney *U* test ($n < 20$ per group) was used to compare mean values, wherever appropriate. Chi-square test was used to compare categorical data. Multivariate analysis was performed using the MANOVA test. An exact two-sided *P*-value of less than 0.05 was considered statistically significant. Correlation between independent donor variables and dependent outcome variables was assessed by linear regression and r^2 Pearson coefficient calculation.

Results

Of 564 procedures performed during the study period, 42 pancreases were processed from donor ≤20 years (7.4%), hereafter referred to as the YD group, and 522 from donors > 20 years (92.6%), hereafter referred to as the OD group. Donor variables are summarized in Table 1. The YD group had lower body mass index (BMI) and a higher male/female ratio compared with OD. The most common cause of death was cerebral trauma in YD and cerebro-vascular disease in OD. Warm and cold ischemia times, duration of ICU stay, and type of preservation solution were similar in both groups.

Islet isolation outcomes are summarized in Table 2. Pancreas weight before digestion was significantly lower in YD. Digestion time was similar in both groups. The proportion of digested tissue (digestion rate) was higher in the YD group. The total volume of compacted tissue after digestion was higher in YD compared to OD. Before purification, islet numbers and IEQ numbers were higher in YD compared to OD, albeit not significantly. The percentage of

Table 1. Characteristics of young donors (≤20 years) and older donors (>20 years).

	Young Donors (≤20 years) (*n* = 42)	Older Donors (>20 years) (*n* = 522)	*P*-value*
Age, years (mean ± SD)	17.8 ± 2.6	48.5 ± 11.3	NA
Age, years (median, min–max)	19 (6–20)	50.5 (21–71)	NA
Sex			
Male (%)	32 (76.2)	307 (58.8)	0.032
Female (%)	10 (23.8)	215 (41.2)	
BMI, kg/m^2	23.5 ± 4.4	26.0 ± 4.5	0.001
ICU stay, days	2.5 ± 1.9	2.4 ± 2.0	0.854
Warm Ischemia Time, min	59 ± 22	67 ± 33	0.163
Cold Ischemia Time, min	332 ± 141	373 ± 161	0.118
Cause of Death			<0.001
Cerebral trauma (%)	29 (69.0)	113 (21.6)	
Cerebro-vascular (%)	6 (14.3)	339 (64.9)	
Suicide (%)	4 (9.5)	29 (5.6)	
Anoxia (%)	2 (4.8)	19 (3.6)	
Others	1 (2.4)	22 (4.2)	
Preservation Solution			0.978
UW (%)	17 (40.5)	220 (42.1)	
IGL-1 (%)	15 (35.7)	175 (33.5)	
Celsior (%)	7 (16.7)	95 (18.2)	
Other (%)	3 (7.1)	32 (6.1)	
Two-layer method			0.306
Yes (%)	5 (11.9)	102 (19.5)	
No (%)	37 (88.1)	420 (80.5)	

BMI, body mass index; ICU, intensive care unit; SD, standard deviation; UW, University of Wisconsin solution; IGL-1, Institut Georges Lopez-1 solution.

*Student *t*-test for continuous variables and chi-square test for binary or categorical variables (global *P*-value).

embedded islets before purification was significantly higher in YD.

After the purification process, both total islet numbers and total IEQ numbers were significantly lower in YD compared to OD. Postpurification IEQ number per gram of pancreas was also lower in YD compared to OD (Fig. 1). Recovery rate, that is, percentage of the islets extracted after digestion that were effectively recovered by purification, was significantly lower in YD. Isolation success (i.e., final yield ≥250 000 IEQ) was similar in the two groups. Finally, the proportion of islet preparations that was clinically transplanted was similar in both groups. In fact, in the YD group, two preparations were transplanted with <250 000 IEQ and one preparation with ≥250 000 IEQ was not transplanted; in the OD group, 25 preparations were transplanted with <250 000 IEQ and 41 preparations with ≥250 000 IEQ were not transplanted.

We attempted to identify which donor factors could impact on islet yields in the YD group. However, in this age group, IEQ number (pre- and postpurification) and recovery rate correlated neither with donor age, body weight or body mass index (Fig. 2).

Quantity and quality assessment was performed in preparations that were transplanted (n = 16 in YD and n = 224 in OD). Characteristics of these islet preparations are shown in Table 3. Transplant volume (compacted) was higher in YD compared to OD, albeit not significantly. Islet viability was similar in both groups. The purity of the preparations was significantly lower in YD compared to OD. Endotoxin levels were lower in YD compared to OD, albeit not significantly. Stimulation index was 50% higher in YD compared to OD but failed to reach statistical significance.

Discussion

Selection of suitable donors is one key to the success of human islet isolation and transplantation. Many donor factors such as age, BMI, cold ischemia time, and cause of death are all identified as affecting the recovery of human islets [4–7,20–22]. According to several studies, the use of pancreases from donors ≤20 years of age is associated with lower islet isolation results, at least in terms of yields [6,7,20]. However, it is important to keep in mind that *in vitro* function of islets from YD has been reported to be significantly better [7], an advantage that may overcome the lower yields observed in these donors. Nonetheless, a recently introduced donor scoring system allocates low points for donors <25 years [5]. On the other hand, only few studies report data on successful isolation and transplantation of islets from YD, and most suggest to adapt the isolation technique to this particular situation [9,20,23,24]. In the present study, the same isolation protocol was used for all age groups. The only differences observed between groups were unsurprisingly associated with young age: There were more male with cerebral trauma in the YD group (related to the mechanism of death in this age group [25]), and YD had lower BMI and pancreas weight [26].

The major obstacle to successful islet isolation from young donor pancreases lies in the difficulty to liberate islets from the exocrine tissue [4,9,23]. The study supports this view by demonstrating that the proportion of embedded islets is higher in YD than in OD. This leads to a significantly lower recovery rate, that is, a smaller proportion of the islets extracted during the digestion phase that we were actually able to purify. Interestingly, both groups had similar islet size before purification, but YD islets were larger after purification. In other words, a loss of smaller islets was observed in the YD group after purification. This was possibly related to the fact that small mantled islets are less susceptible to escape migration with the exocrine tissue

Table 2. Comparison of outcomes of the islet isolation procedure for young donors (≤20 years) and older donors (>20 years).

	Young donors (≤20 years) (n = 42)	Older donors (>20 years) (n = 522)	P-value (univariate)*	P-value (multivariate)†
Pancreas weight, g	83.0 ± 19.4	99.3 ± 23.7	<0.001	<0.001
Undigested tissue weight, g	6.1 ± 4.9	18.5 ± 12.3	<0.001	<0.001
Digested tissue weight, g	75.5 ± 18.5	80.7 ± 22.4	0.167	ND
Digestion rate, %	92.5 ± 5.6	81.4 ± 11.4	<0.001	<0.001
Digestion time, min	17 ± 4	18 ± 5	0.149	ND
Tissue volume, ml	52.8 ± 15.9	41.6 ± 15.5	<0.001	<0.001
Total number of islets, prepurification	566 797 ± 343 675	450 541 ± 384 551	0.067	ND
IEQ, prepurification	407 429 ± 233 699	341 421 ± 166 926	0.020	0.93
IEQ per g pancreas (prepurification)	5598 ± 3481	4389 ± 2152	0.002	0.54
Mean prepurification islet size, μm	135.7 ± 99.2	131.9 ± 121.2	0.850	ND
Embedded islets, %, prepurification	44.3 ± 22.7	24.9 ± 20.9	<0.001	<0.001
Fragmented islets, %, prepurification	12.3 ± 8.7	14.6 ± 10.0	0.260	ND
Total islets postpurification	199 553 ± 168 658	316 157 ± 199 305	<0.001	<0.001
IEQ postpurification	180 982 ± 128 073	244 167 ± 134 137	0.003	0.006
IEQ/per g pancreas (postpurification)	2412 ± 1789	3194 ± 1892	0.013	0.01
Mean postpurification islet size, μm	173.7 ± 93.4	133.9 ± 67.3	0.001	0.004
Recovery rate, %	47.8 ± 30.4	76.2 ± 37.4	<0.001	0.002
Isolation success (i.e., final yield ≥250 000 IEQ) (%)	15 (35.7)	240 (46.0)	0.259	ND
Transplanted preparation (%)	16 (38.1)	224 (42.9)	0.628	ND

IEQ, islet equivalent; ND, not performed.

*Student t-test for continuous variables and chi-square test for binary or categorical variables (global P-value).

†Only those variables with significant differences in the univariate analysis were entered in the multivariate model. P-values were computed using MANOVA analysis.

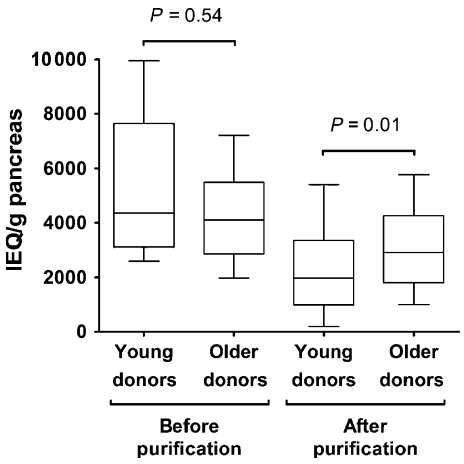

Figure 1 Islet equivalent (IEQ) per gram pancreas before and after purification in young donors and older donors. Box and whisker plots are shown, showing, respectively, first and third quartiles, and 10th and 90th percentiles.

during the purification process compared to large islets. It must also be noted that *in vitro* function, assessed by glucose-stimulated insulin release, was 50% higher in YD compared to OD. Low numbers in the YD group and high variability (standard deviation) likely explain why such differences failed to reach statistical significance.

It is therefore obvious that islets from YD show a significantly lower recovery rate because of this inherent difficulty in separating embedded islets from exocrine tissue during the purification step. In contrast to what is observed for adult donors, difficulty in isolating juvenile or pediatric islets has no relationship to body weight or BMI. Research efforts should be directed at improving the digestion step, possibly through the use of different neutral protease to collagenase [27] or tryptic-like activity to collagenase [28] ratios in the enzyme blend and modified digestion protocols [24]. The sequential use of collagenase and neutral protease could also improve the recovery rate [29]. Improving the recovery of exocrine-attached islets after purification could also be achieved by developing protocols integrating an additional "rescue" purification step of the islets trapped in exocrine tissue [30]. Another challenge is to improve our knowledge of structural tissue characteristics that render islet isolation in young donors so challenging. Better understanding of the extracellular tissue matrix composition within the islet-exocrine interface is needed to improve current digestion protocols [31]. Finally, the type of enzyme blend utilized for the digestion process could be assumed to have an impact, but we found no significant differences in an analysis of islet yield, percentage of embedded islets, and islet recovery rate according to enzyme blend in either donor age group (data not shown).

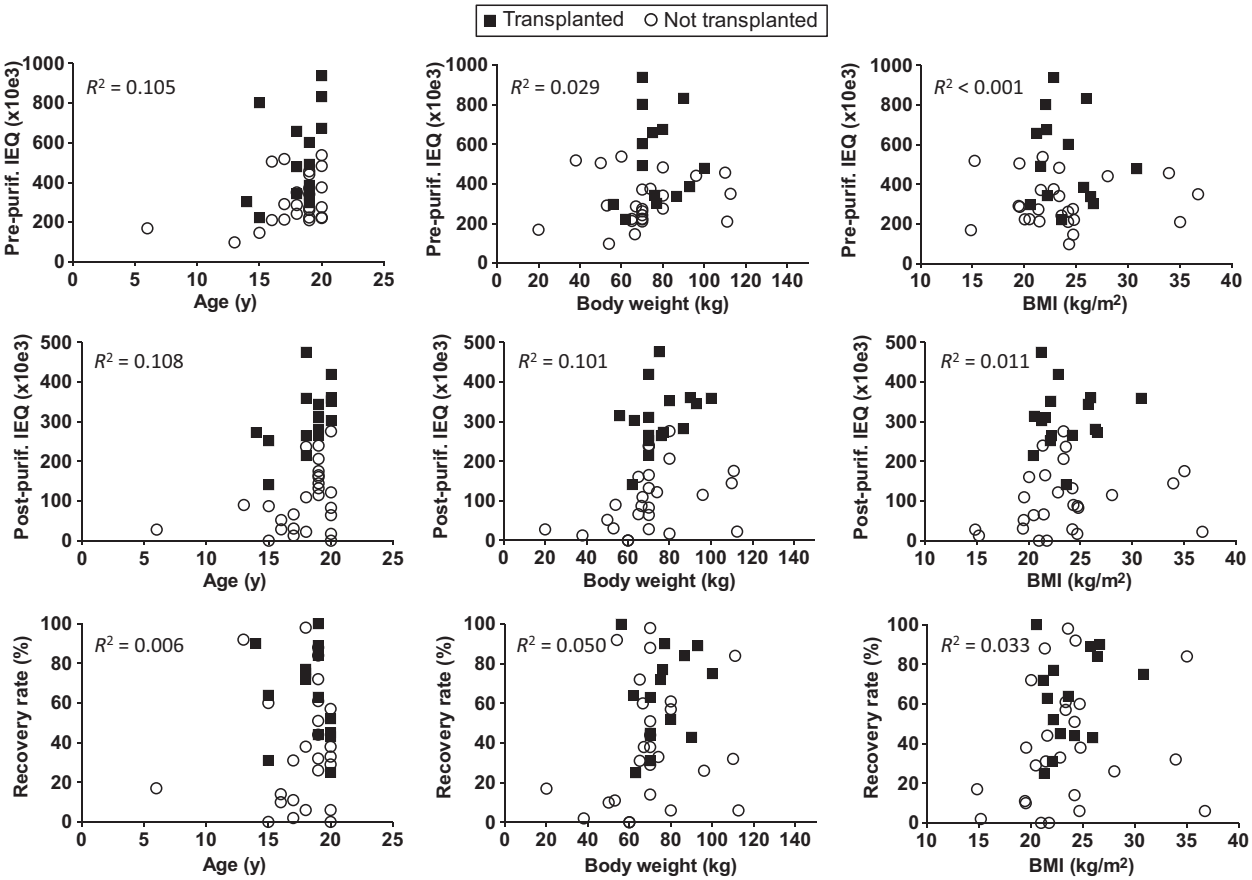

Figure 2 Pre- and postpurification islet equivalent (IEQ) number, and recovery rate (dependent variables) as a function of age, body weight, or body mass index (BMI) in young donors (independent variables). Pearson coefficients (r^2) calculated by linear regression are shown for each set of variables.

Table 3. Quality control data of islet preparations in young donors (≤20 years) and older donors (>20 years).

	Young donors (≤20 years) (n = 16)	Older donors (>20 years) (n = 224)	P-values*
Packed transplant volume (ml)	4.1 ± 3.2	2.8 ± 1.5	0.173
Viability (%)	90.8 ± 4.7	90.8 ± 4.6	0.890
Purity (%)	51.0 ± 19.5	59.6 ± 15.9	0.037
Endotoxin contents (EU/ml)	0.24 ± 0.22	0.47 ± 1.6	0.096
Stimulation index	4.5 ± 5.1	3.0 ± 5.7	0.350

*Mann–Whitney *U* test.

In spite of the loss of islets during purification, there was no significant difference in the rate of transplanted preparations between YD and OD. Considering that transplanted islet number is poorly correlated with islet graft function [8], the commonly used threshold of 5000 IEQ/kg body weight per infusion [1,2] could probably be lowered to a significant extent when transplanting islets isolated from YD.

One limitation of the study resides in the low numbers of cases in the YD group. However, it must be emphasized here that this is not the result of hyperselection of juvenile or pediatric donor offers. Encouraged by the good isolation/transplantation outcomes achieved, our threshold for acceptance is in fact very low. The major reasons are the low number of pediatric organ donors and the high acceptance rate of such organs for whole-pancreas transplantation, favored by the current organ allocation schemes that prioritize whole pancreas over islets [8,32].

Taken together, these data show that, in spite of the known difficulties in extracting islets from YD, a high rate of transplantable islet preparations with superior function can be obtained with these donors. The critical step of extracting islets from the surrounding exocrine tissue during the digestion phase should be a focus of research. In spite of isolation and purification difficulties, pancreases from YD allowed similar islet transplantation rates as OD. Efforts should be directed at improving islet extraction in

these donors to realize their full potential for islet transplantation.

Authorship

IS, RM, and TB: designed the study; IS, RM, YM, SB, LB, and DB: collected the data; RM, IS, LB, CT, and TB: analyzed the data; RM and IS: performed statistical analysis; RM, IS, PM, and TB: wrote the article; TB: full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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