Sodium Level of Human Pancreatic Donors is a Critical Factor for Determination of Islet Efficacy and Survival

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Running title

Human islets from hypernatremic donors
Keywords

Hypernatremia, islet isolation and transplantation, NOD Scid mice, sodium chloride, ion channels.

Abbreviations

BMI: Body mass index
DTZ: Dithizone
GSIS: Glucose stimulated insulin secretion
IEQ: Islet equivalent
LSC: Laser Scanning Cytometry
NOD Scid: Non-obese diabetic (severe combined immune deficiency)
STZ: Streptozotocin
TUNEL: Terminal deoxynucleotide transferase-mediated dUTP nick-end labeling
Abstract

Organs from hypernatremia (elevated Na⁺) donors when used for transplantation have had dismal outcomes. However, islet isolation from hypernatremic donors for both transplantation and research applications has not been investigated yet. A retrospective analysis of in vivo and in vitro islet function studies was performed on islets isolated from hypernatremic (serum sodium levels ≥ 160 mEq/L) and normal control (serum sodium levels ≤ 155 mEq/L) donors. Twelve isolations from 32 hypernatremic and 53 isolations from 222 normal donors were randomly transplanted into diabetic NOD Scid mice. Sodium levels upon pancreas procurement were significantly elevated in the hypernatremia group (163.5 ± 0.6 mEq/L) as compared to the normal control group (145.9 ± 0.4 mEq/L) \( (P<0.001) \). The post-culture islet recovery rate was significantly lower in the hypernatremia (59.1 ± 3.8%) group compared to the normal (73.6 ± 1.8%) group \( (P=0.005) \). The duration of hypernatremia was inversely correlated with the recovery rate \( (R^2=0.370, \ P<0.001) \). Furthermore, the percentage of successful graft function when transplanted into diabetic NOD Scid mice was significantly lower in the hypernatremia (42%) group in comparison to the normal control (85%) group \( (P<0.001) \). The ability to predict islet graft function post transplantation using donor sodium levels and duration of hypernatremia was significant \( \text{(ROC analysis, } P=0.022 \text{ and 0.042, respectively)} \). In conclusion, duration of donor hypernatremia is associated with reduced islet recovery post culture. The efficacy of islets from hypernatremia donors diminished when transplanted into diabetic recipients.
Introduction

The acute shortage of donor pancreata has increased the use of pancreata from marginal donors in order to meet the growing demand for clinical islet transplantation (20, 43, 46). Hence, it is important to evaluate the characteristics of donors which may influence the outcomes of islet isolation (36). According to published studies, critical donor factors that affect islet isolation include age (31, 35, 52, 57), cause of death (16, 31, 57), cold ischemia time (CIT) (7, 10, 41), body mass index (BMI) (9, 20, 26, 54), and the acute insulin response to arginine (22). The aforementioned factors have been widely reported, however, donor hemodynamic and biochemical parameters have not been thoroughly investigated (14). Specifically, the association between donor serum sodium levels and islet isolation outcomes is yet to be analyzed.

Hypernatremia is commonly seen in brain injury patients (48, 50), especially those who develop central diabetes insipidus (DI) and/or over usage of osmotic diuretics such as mannitol (6, 18). As a result of DI, probable impairment of kidney function and production of large amounts of unconcentrated urine leads to hypernatremia and increased plasma osmolality (30). Therefore, a sustained hypernatremia state in brain dead donors eventually cause cellular dehydration (55).

It has been reported that uncorrected hypernatremia in whole organ donors is associated with poor graft or patient survival (3, 15, 17, 21, 25). Totsuka et al. not only provided supporting evidence for the above results, but also found that hypernatremia in donor corrected to 155 mEq/L or less before procurement did not adversely impact on liver
graft survival (53). Furthermore, in Totsuka’s study, deteriorated liver graft function after transplantation was explained by sudden changes of extracellular osmolality in a liver graft obtained from a donor with hypernatremia could cause hepatocyte injury (53). In contrast to solid organ transplantations, islet transplantation requires the isolation of islets from brain-dead donor pancreata before infusion. A literature search indicated that the relationship between donor serum sodium levels and islet isolation outcome has not been fully investigated. Hence, we hypothesize that hypernatremia of deceased donors prior to procurement may influence islet survival and function post isolation. To test this hypothesis, a retrospective analysis of the results of islets in hypernatremic brain-dead donors was performed.

Materials and Methods

Donor groups

Donor demographic information and human islet isolation data (n=254) were obtained from the human islet manufacturing database of the City of Hope. Donors with elevated Hemoglobin A1c (HbA1c ≥ 6.5%), DCD criteria, and cardiac down time were all excluded from this analysis. Serum sodium levels were extracted from donor charts originally provided by organ procurement organization (OPO). Sodium levels at admission, at its highest during hospitalization, and prior to procurement for each donor were documented. In this study, two groups of donors were designated based on the last recorded sodium levels prior to procurement. Donors with sodium levels of 155 mEq/L or less were categorized as the normal control group (n=222) and those with serum sodium levels of 160 mEq/L or greater were considered the hypernatremia group (n=32). The
duration of hypernatremia was calculated based on the time between the first recorded reading of sodium levels >160 mEq/L until the last reading right before procurement.

**Islet isolation and in vitro assessment**

Pancreata from brain-dead donors were procured, stored in cold preservation solution, and transported to the human islet isolation facility at City of Hope following standard protocol. Pancreatic islets were isolated following COH method (23), which is a modification of the method described by Ricordi et al (45). After pancreas cleaning, the cannulated pancreas was perfused with Collagenase enzyme solution using an automated perfusion machine (BioRep Tech. Miami, FL, USA). The distended pancreas was then cut into 7-10 pieces and transferred to the Ricordi digestion chamber. After transferring the pancreatic tissue, the chamber was warmed up to 37°C for digestion. During the pancreas digestion process, the chamber was shaken to mechanically disrupt the tissues to liberate the islets. Tissue samples were taken every minute and stained with Dithizone (DTZ) (Sigma Aldrich, St. Louis, MO, USA) (32); once 50% of free islets were detected under the microscope, the digestion phase was stopped and dilution phase was initiated. The time between the start of digestion and the initiation of dilution is defined as the digestion switch time. Digested tissue was collected, washed, and pooled in media supplemented with human serum albumin. Samples were taken for pre-purification islet count from pooled digested tissue. Islet purification was done with Biocoll Separation Solution (Biochrom AG, Cedarlane Laboratories, Canada) using continuous density gradients (23). Purified islets were pooled based on purity into different fractions. Post-purification samples were subsequently taken from each fraction to acquire islet counts.
for each fraction, expressed as IEQ using previously described methods (44). Recovery rate of islets after purification was calculated based on following formula: recovery rate post purification (%) = (total IEQ number post-purification / total IEQ pre-purification) × 100. Isolated islets were then cultured in CMRL-1066 supplemented culture media as described previously (19) (Mediatech, Manassas, VA, USA) at 37 °C/5% CO₂ for further assessment.

Islets were cultured for 24-72 hours prior to assessment. Islet count and viability was determined using DTZ and fluorescein diacetate and propidium iodide (Sigma-Aldrich, St Louis, MO, USA) respectively following the methods previously described (4). The recovery rate post culture was calculated: recovery rate post culture (%) = (total IEQ number after culture / total IEQ post-purification) × 100. Glucose stimulated insulin secretion (GSIS) was performed using static incubation or perifusion assay (5, 23). Assessments of cultured islets were conducted independently by quality control team.

Quantification of beta and apoptotic beta cells using Laser Scanning Cytometry (LSC)

Islets from hypernatremia (n=20) and control (n=20) groups pre- and post-culture were processed for LSC analysis using our previously described method (51). Briefly, aliquots of 1,000 IEQ human islets were fixed with 10% formalin and paraffin blocks were prepared. Sections were dehydrated and processed for immunofluorescent staining. Sections were then treated overnight at 4°C with poly-clonal Guinea Pig anti-insulin (DAKO Cat. #A0564) at 1:100 dilution. Secondary antibody was Donkey anti-Guinea
Pig Texas Red + Cy5 at 1:100 dilution. Apoptotic cells were assayed using Takara kit (Cat.# MK500, Clontech Laboratories, Inc. Mountain View, CA, USA) for detection of early apoptotic cells using the modification of the method described by the manufacturer. The sections were stained for 10 min with DAPI (0.2 μg/mL) (Sigma, St Luis, MO, USA). The slides were scanned using an iCys laser scanning cytometer (ThorLabs, Newton, NJ, USA) as described previously (51). LSC was conducted using the 405-, 488- and 633-nm lasers for excitation and the iCys 3.4 software for analysis. Scanning and analysis of each sample were performed on at least two randomly selected sections, covering the entire sections containing 3,000 to 30,000 cells. Percentage of beta cells was expressed by the percentage of insulin-positive cells in each preparation. Percentage of apoptotic beta cells was expressed by percentage of insulin and TUNEL double positive cells in each preparation (51).

Islet transplantation in diabetic NOD Scid mice

All the procedures involving animals were approved by the Institutional Animal Care and Use Committee of the City of Hope. All mice were transplanted by a single well-trained individual for consistency and reproducibility of the results. Islet assessment for quality control was carried out in NOD Scid mice for islets isolated for clinical transplantation and research applications. The islet number (1,200 IEQ) transplanted per mouse in our center is established as an optimum islet mass. The culture period (24-72 hrs) for the islets from two donor groups were similar. Typically three NOD Scid mice (male, 10-12 weeks of age) (Jackson Laboratory, Bar Harbor, ME, USA) were used as recipients for transplantation of isolated human islets. Diabetes was induced in mice by
intraperitoneally injecting 50 mg/kg streptozotocin (STZ; Sigma-Aldrich, St Louis, MO, USA) for three consecutive days. The mice with hyperglycemia (> 350 mg/dL) for at least two consecutive days were used as recipients. Diabetic NOD Scid mice were transplanted with an identical number of human islets from the same donor under the left kidney capsule. Diabetes reversal was monitored 2-3 times per week for 30 days to measure blood glucose levels using a glucometer (LifeScan, Inc., Milpitas, CA, USA). Successful graft function was achieved if two-thirds of transplanted mice were normoglycemic (< 200 mg/dL) within 14 days and maintained normoglycemia levels for more than 20 days. Poor graft function was determined if above criteria was not met.

**Statistical analysis**

GraphPad Prism (GraphPad Software 6.0, La Jolla, CA, USA) was used to analyze the data and generate the figures. Unpaired Student’s t test (two-tailed) or Fisher’s exact test was used to compare between the normal control and hypernatremia groups. Values were expressed as mean ± standard error of mean (SEM). Linear regression analysis was used to determine the degree of correlation between the duration of hypernatremia and islet recovery rate post culture. For evaluation of the ability of multiple parameters to predict transplant efficacy, Receiver Operating Characteristic (ROC) curves were generated using GraphPad Prism. ROC curves plots of the sensitivity (true positives/[true positives + false negatives]) versus 1-specificity (true negatives/[true negatives + false positives]) (1, 24). The ROC analysis calculates the area under the curve (AUC) to show predictability of the test. For AUC of 0.5, there is no predictive relationship; an AUC of 1 is considered perfectly predictive. In our study, the AUC was used to predict islet graft
function based on donor sodium levels or duration of hypernatremia. For all the tests used, $P<0.05$ was considered significant.

**Results**

Donor demographic information is listed in Table 1. Thirty-two out of 254 donors (12.6%) presented elevated serum sodium levels $\geq 160$ mEq/L, designated as the hypernatremia group, and the normal control group (n=222) exhibited serum sodium levels $\leq 155$ mEq/L prior to procurement. There was no significant difference in serum sodium levels at admission between the two donor groups and both were within normal range ($\leq 155$ mEq/L). However, the peak sodium level in hypernatremia group ($167.9 \pm 0.9$ mEq/L) was significantly higher than that of the normal control group ($152.8 \pm 1.3$ mEq/L) ($P<0.001$). As expected, the last records of serum sodium levels before procurement was significantly increased in hypernatremia group ($163.5 \pm 0.6$ mEq/L) compared to the normal control group $145.9 \pm 0.4$ mEq/L ($P<0.001$). No significant differences were observed between hypernatremia and normal control groups in terms of age, gender, and BMI. In regards to cause of death, more than half of the donors in both groups suffered from cerebrovascular accidents (hypernatremia group: 59%; normal control group: 53%). Similar rates of head trauma were also observed between the two groups (hypernatremia group: 22%; normal control group: 33%).

Table 2 shows the results of islet isolation and in vitro assessments. Cold ischemia time was not significantly different between the two groups. The switch times for pancreatic digestion were similar, indicating that high serum sodium levels did not affect the
digestion of the pancreata. Total IEQ pre-purification, post purification, and post culture were also similar between the two groups. Islet yields (IEQ/g of pancreatic tissue) post purification and post culture also did not show significant differences. Furthermore, there were no significant differences when islet yields were analyzed by fractions. However, the recovery rate of islets post culture was significantly lower in hypernatremia group (59.1 ± 3.8%) compared to the normal control group (73.6 ± 1.8%) ($P=0.005$). In the hypernatremia group, the average hypernatremia duration was 23 hrs (range 4-48 hrs). Duration of hypernatremia was inversely correlated with the islet recovery rate post culture ($R^2=0.370$, $P<0.001$) (Fig. 1). The recovery rate of islets during purification was not significantly different. Post-culture islet viability and GSIS, the results showed lower glucose-stimulated insulin release in hypernatremia donor groups compared to normal control group, albeit the differences were not significant.

LSC analysis showed that percentage of beta cells in the hypernatremia group was 42.36 ± 2.51 %, which was not significantly different as compared to that in the normal group (44.88 ± 1.92 %) ($P=0.426$). Similarly, the percentage of apoptotic beta cells was not significantly different between the hypernatremia and normal control groups (2.90 ± 0.63 % vs. 2.88 ± 0.30 %) ($P=0.977$) (Fig. 2).

Islets from 12 hypernatremia and 53 normal control donors were randomly transplanted into STZ induced diabetic NOD Scid mice and followed for 30 days to evaluate graft function. As noted in Table 3, there were no significant differences regarding donor characteristics between the hypernatremia and the normal groups that were transplanted.
The percentage of successful graft function was significantly lower in the hypernatremia (5/12 isolations, 42%) group in comparison to the normal control (45/53 isolations, 85%) group (*P*<0.001) (Fig. 3). Figure 3 shows the ability to predict successful or poor graft function in NOD Scid transplanted mice for each isolation using donor sodium levels. This was statistically significant using ROC analysis: area under the curve (AUC) =0.697 (95% CI: 0.538-0.857, *P*=0.022) (Fig. 4). The optimal cut-off point for donor sodium level was >158 mEq/L, with 47% sensitivity and 92% specificity. For further analysis of glycemic control, AUC of blood glucose levels over 200 mg/dL during the follow-up of 30 days was compared between the hypernatremia and normal control groups. The AUC for the hypernatremia group (3443 ± 1068) was significantly higher than that in the normal control group (1083 ± 265) (*P*=0.019) (Fig. 5), indicating diminished islet function in glycemic control as a result of hypernatremia.

Furthermore, the results of ROC analysis showed that the graft function could be predicted by duration of hypernatremia (AUC=0.896; 95% CI: 0.682-0.110; *P*=0.042) (Fig. 6). The optimal cut-off points for duration of hypernatremia was >18.5 hrs (83% sensitivity and 100% specificity).

**Discussion**

Brain-dead donors often develop severe diabetes insipidus (DI), which has been linked to hypernatremia, particularly at the time of organ procurement (6, 47). Sodium levels greater than 145 mEq/L is defined as hypernatremia in patients with severe traumatic brain injury (29). However, patients with potential to be a donor for organ procurement
for transplant application, hypernatremia of $\text{Na}^+ \geq 160 \text{mEq/L}$ negatively affects outcome of solid organ transplantation (15, 17, 21, 25, 53). It is not clear why this discrepancy of sodium levels between patients with severe traumatic brain injury and those donors selected for organ procurement exists. Nevertheless, selection of organs with $\text{Na}^+ < 160 \text{mEq/L}$ would result in excluding many donors for organ recovery. Therefore, in this study we chose sodium levels of 160 mEq/L as the hypernatremia threshold. Hoefer et al. hypothesized that donor hypernatremia may aggravate the existing reperfusion injury caused by intracellular sodium increase due to acidosis and $\text{H}^+$/Na$^+$ exchange during ischemia and cellular Ca$^{2+}$ overload due to the Ca$^{2+}$ influx (21). Gonzales et al. reported that livers obtained from hypernatremic donors when transplanted into recipients resulted in poor graft functions, suggesting that increased intracellular osmolality affects graft outcome (11, 17). Although it is not clear why hypernatremic organs function poorly when transplanted, it is probable that the biophysiology to maintain cell integrity is critical to determine transplantation outcomes. It has been suggested that an elevated sodium concentration intracellularly may cause an increase of osmolality and this in turn resulted in hepatocyte injury and liver obtained from donors with hypernatremia failed to function when transplanted (53). Thus, correction of hypernatremia prior to procurement resulted in improvement of liver function (53). In contrast to solid organ transplantation, the impact of hypernatremia on islet isolation and transplantation outcomes has not been investigated. In this study, we report that donor hypernatremia ($\geq 160 \text{mEq/L}$) at procurement has a major impact on islet functional quality, particularly when transplanted into diabetic NOD Scid mice. The reversal rate of diabetes in transplanted NOD Scid mice from the hypernatremia group was significantly lower than in the normal
control group. In fact, unstable and fluctuating blood glucose levels were observed in mice that received islets from hypernatremia donors.

In this retrospective analysis, a total of 254 islet isolations were carried out, of which approximately 13% of the donors exhibited serum sodium levels ≥ 160 mEq/L. Despite elevated sodium levels, differences in islet yield pre- and post-purification and islets recovered from purification were not statistically significant between the two groups. This indicates that elevated sodium levels at this stage may not critically influence islet isolation outcomes. However, it is worth noting that islet recovery rate post culture in the hypernatremia group was significantly lower than that in the normal control group. It is conceivable that cellular damage of the islets cultured intensified during the culture period from the donors with high serum sodium levels. Most recently, a study showed that Na\(^+\) channel blocker (Ranolazine) inhibit glucagon release from pancreatic α cells and reduce glucose levels in diabetic animals (13). In another study, Anazawa et al. used chloride channel inhibitors during the rodent pancreatic digestion with collagenase for islet isolation, which significantly improved islet recovery and function (2). Additionally, using sodium/chloride free solution during the digestion process has also resulted in improvement of islet yield suggesting that the presence of excess chloride influences islet yield and function after transplantation into diabetic SCID mice (2). They also suggested that islet cell death was the result of chloride influx into the cells and subsequent damage on cell membrane integrity due to disruption of lysosomes (2). Influx of chloride ions into cells may exacerbate cell death when transitioning from dehydration (hypernatremia before procurement) to normal osmolality during isolation/culture of
islets, which causes more cell death and impaired function (11). Interestingly, in our study, chloride levels in hypernatremic donors were also elevated (data not shown), suggesting that the influx of chloride ions may also trigger cell death hence islet loss post culture.

It has been reported that changes in activities of various ion channels play important roles in apoptotic, necrotic or ischemic cell death (33, 37-39). Previous studies have shown that hypernatremia reduce T-type Ca\(^{2+}\) channel resulted in stimulation cyto-protective protein kinase pathway (40). Furthermore, it has also been reported that high salt concentration activates both inflammatory pathway and classic hypertonicity induced p38/MAPK pathway (28). In our study using TUNEL assays, apoptotic beta cells were not significantly different between hypernatremia and control groups, suggesting that alternative programmed cell death of hypernatremic islets may be present. Studies have shown that when insulinoma cell line (HIT) incubated in isotonic solution in the presence of Na\(^+/\)K\(^+\)/2Cl\(^-\) transport blocker, Furosemide causes cellular shrinkage (27). However, cell swelling has been induced in a hypotonic solution (27). In this study, we have shown that islets from hypernatremic donors, when cultured in vitro, resulted in significant islet loss. It is not clear why this significant loss occurs, but it is conceivable that several different solutions used throughout the isolation process including: cold pancreas preservation solution (UW, HTK, SPS-1), pancreas trimming solution, perfusion enzyme solution, digestion solution, wash solution, and lastly density gradient purification solutions may impact islet isolation outcomes, especially those islets from hypernatremia donors. The entire process of islet isolation is carried out at a relatively low temperature.
(4°C), which theoretically ion channels are inactive. However, since the digestion process with enzymes and mechanical dissociation to free the islets is carried out at 37°C, whereby the ions channels could be actively affecting ion influx, possibly influencing islet function, integrity, and survival (8). Thus, islets exposed to various solutions with different ionic mechanisms may influence intra/extra-cellular osmolality (56); which in turn may cause cellular shrinking or swelling affecting islets.

Previous studies have shown that the use of anti-diuretic agent, Vasopressin, in DI donors prevented excessive water loss and sodium elevation in donors (53). However, this treatment may not be suitable for pancreata for islet isolation since Vasopressin has been reported to cause ischemia of the pancreas, thus alternative methods are needed to mitigate hypernatremia in donors (53). It is not clear why islets from hypernatremic donors display normal in vitro function yet when transplanted, the reversal of diabetes was dismal. In vivo islet survival and function depends on complex electrical signal systems namely the influx of Na⁺, K⁺, Ca²⁺, and Cl⁻ across the cell membrane. The study of in vivo mechanisms of ion channels is technically challenging, however investigating islet transplantation from hypernatremic donors into anterior chamber of eye in live animals may unravel islet function and vascularization from hypernatremic donors (34, 49).

The biochemical profiles of donors have recently been shown to be important parameters to determine islet isolation outcomes as well (31). In particular, it has been reported recently that HbA1c has detrimental effects on isolation outcomes (12, 42). In this study,
elevated Na\textsuperscript{+} at the time of pancreas procurement resulted in decline of islet transplant outcomes in immune-deficient diabetic NOD Scid mice, especially these donors with >18.5 hrs of sustained hypernatremia and islet loss >28% post culture. We retrospectively analyzed 32 isolations from the hypernatremia group; islets from only one donor from this group were transplanted into type 1 diabetic recipient. The last sodium level pre-procurement for this particular donor was 165 mEq/L. Although the islet morphology was excellent, the islet recovery rate post culture was 52%, which was lower than the average in the hypernatremic group (59.1\% \pm 3.8). Consequently, minimum insulin reduction was achieved when these islets were transplanted. The result may suggest that parameters for donor selection, including biochemical parameters, are important for success of clinical islet transplantation.

In conclusion, donor hypernatremia is associated with a significant islet loss post culture and the function diminished when transplanted into recipients.

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Disclosures

All authors declare that there is no duality of interest associated with this manuscript.

Contribution statement

MQ contributed to study design, data collection and analysis, and manuscript writing and revision. LV and SB contributed to data collection and assembly, data analysis, and manuscript revision. KO, JR, BM, JJ, IN, YM, DD, and ME contributed to data analysis and interpretation, and manuscript revision. FK and IHA contributed to study conception and design, data interpretation, and to manuscript writing and revision. All authors approved the final version.
References


Figure legends

Figure 1
Association between duration of hypernatremia and post-culture islet recovery rate. The duration of hypernatremia was inversely correlated with the post-culture islet recovery rate (n=27) (p<0.001).

Figure 2
Percentage of beta cells (A) and apoptotic beta cells (B) for two donor groups. Laser Scanning Cytometry analysis showed that percentage of beta cells in hypernatremia group was 42.36 ± 2.51 %, which was not significantly different as compared to that in normal group (44.88 ± 1.92 %) (P=0.426). Moreover, the percentage of apoptotic beta cells was not significantly different between hypernatremia and normal control group (2.90 ± 0.63 % vs. 2.88 ± 0.30 %) (P=0.977).

Figure 3
Comparison of successful islet graft function between the two donor groups. Successful graft function was achieved if two-thirds of transplanted mice were normoglycemic (<200 mg/dL) within 14 days and maintained normoglycemia levels for more than 20 days. Lower frequency of successful graft function was achieved in the hypernatremia group (5/12 isolations, 42%) in comparison to the normal control group (45/53 isolations, 85%) (*P<0.001).
Predictive power of donor sodium levels on islet graft function. Receiver Operating Characteristic analysis shows the significant predictability of successful or poor graft function in NOD Scid mice for each islet isolation (AUC=0.697; 95% CI: 0.538-0.857; \(P=0.022\)). The optimal cut-off point for donor sodium level of \(>158\) mEq/L had 47% sensitivity and 92% specificity.

Area Under Curve (AUC) of blood glucose (BG) levels over 200 mg/dL in transplanted mice during the follow-up of 4 weeks. AUC levels were significantly higher in the hypernatremia group (3443 ± 1068, n=12) as compared to normal control group (1083 ± 265, n=53) (*\(P=0.019\)).

ROC curve for predicting islet graft function. Successful or poor graft function in NOD Scid mice could be predicted based on duration of hypernatremia (AUC=0.896; 95% CI: 0.682-0.110; \(P=0.042\)). The optimal cut-off points for duration of hypernatremia of \(>18.5\) hrs had 83% sensitivity and 100% specificity.
Table 1. Demographic information of two donor groups identified by last serum sodium levels prior to procurement

<table>
<thead>
<tr>
<th></th>
<th>Sodium level ≥ 160 mEq/L</th>
<th>Sodium level ≤ 155 mEq/L</th>
<th>P value</th>
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<tr>
<td>Number of donors</td>
<td>32</td>
<td>222</td>
<td>N/A</td>
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<td>Donor serum sodium levels (mEq/L)</td>
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<td>Admission sodium levels</td>
<td>141.1 ± 0.9</td>
<td>140.7 ± 0.8</td>
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<td>Highest sodium levels</td>
<td>167.9 ± 0.9</td>
<td>152.8 ± 1.3</td>
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<td>Last levels before procurement</td>
<td>163.5 ± 0.6</td>
<td>145.9 ± 0.4</td>
<td>&lt; 0.001</td>
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<tr>
<td>Donor age (yrs)</td>
<td>45.4 ± 1.6</td>
<td>44.5 ± 0.9</td>
<td>0.716</td>
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<tr>
<td>Donor gender, male % (female %)</td>
<td>69 (31)</td>
<td>58 (42)</td>
<td>0.142a</td>
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<td>BMI (kg/m2)</td>
<td>30.8 ± 1.0</td>
<td>29.8 ± 0.4</td>
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<td>Donor cause of death (%)</td>
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<tr>
<td>CVA</td>
<td>59</td>
<td>53</td>
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<tr>
<td>Head trauma</td>
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<td>33</td>
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<tr>
<td>Other</td>
<td>19</td>
<td>14</td>
<td>N/A</td>
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<tr>
<td>Brain death duration (days)</td>
<td>1.4 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>0.140</td>
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SEM, standard error of mean; BMI, body mass index; CVA, cerebrovascular accident

*a Fisher’s exact test was used for this category variable
Table 2. Isolation results of two donor groups identified by last serum sodium levels prior to procurement

<table>
<thead>
<tr>
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<th>Sodium level ≤ 155 mEq/L</th>
<th>P value</th>
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<td>Pancreata cold ischemic time (hrs)</td>
<td>7.5 ± 0.5</td>
<td>8.2 ± 0.2</td>
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<td>Digestion switch time (min)</td>
<td>11.6 ± 0.5</td>
<td>11.7 ± 0.2</td>
<td>0.820</td>
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<tr>
<td>Total islet yield pre-purification (IEQ)</td>
<td>346,446 ± 26,098</td>
<td>354,642 ± 12,867</td>
<td>0.817</td>
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<td>Total islet yield post-purification (IEQ)</td>
<td>258,868 ± 24,740</td>
<td>252,769 ± 10,546</td>
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<td>213,061 ± 22,813</td>
<td>207,794 ± 9,362</td>
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<td>94,569 ± 18,491</td>
<td>92,388 ± 7,024</td>
<td>0.918</td>
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<tr>
<td>Total islets recovered from purification (%)</td>
<td>75.4 ± 5.5</td>
<td>73.5 ± 1.9</td>
<td>0.770</td>
</tr>
<tr>
<td>Total islet yield post-culture (IEQ)</td>
<td>160,001 ± 17,819</td>
<td>176,178 ± 8,210</td>
<td>0.486</td>
</tr>
<tr>
<td></td>
<td>139,051 ± 16,450</td>
<td>147,614 ± 7,221</td>
<td>0.684</td>
</tr>
<tr>
<td></td>
<td>31,566 ± 6,802</td>
<td>39,931 ± 3,475</td>
<td>0.432</td>
</tr>
<tr>
<td>Total islets recovered from culture (%)</td>
<td>59.1 ± 3.8</td>
<td>73.6 ± 1.8</td>
<td>0.005</td>
</tr>
<tr>
<td>Total islet yield post-purification (IEQ/g)</td>
<td>2,801 ± 338</td>
<td>2,743 ± 116</td>
<td>0.864</td>
</tr>
<tr>
<td>Total islet yield post-culture (IEQ/g)</td>
<td>1,724 ± 238</td>
<td>1,902 ± 88</td>
<td>0.484</td>
</tr>
<tr>
<td>Islet viability (%)</td>
<td>93.3 ± 1.1</td>
<td>94.4 ± 0.4</td>
<td>0.307</td>
</tr>
<tr>
<td>Glucose stimulated insulin secretion (GSIS)(SI)</td>
<td>3.5 ± 0.3</td>
<td>3.9 ± 0.2</td>
<td>0.369</td>
</tr>
</tbody>
</table>
Table 3. Donor characteristics of two groups that islets were randomly transplanted into diabetic NOD Scid mice

<table>
<thead>
<tr>
<th></th>
<th>Sodium level ≥ 160 mEq/L</th>
<th>Sodium level ≤ 155 mEq/L</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of donors</td>
<td>12</td>
<td>53</td>
<td>N/A</td>
</tr>
<tr>
<td>Donor serum sodium levels (mEq/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admission sodium levels</td>
<td>140.8 ± 1.5</td>
<td>141.0 ± 0.6</td>
<td>0.927</td>
</tr>
<tr>
<td>Highest sodium levels</td>
<td>165.8 ± 1.1</td>
<td>151.9 ± 0.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Last levels before procurement</td>
<td>163.0 ± 1.1</td>
<td>145.5 ± 0.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Donor age (yrs)</td>
<td>42.3 ± 2.5</td>
<td>46.1 ± 1.9</td>
<td>0.353</td>
</tr>
<tr>
<td>Donor gender, male % (female %)</td>
<td>75 (25)</td>
<td>62 (38)</td>
<td>0.067&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>29.5 ± 1.4</td>
<td>29.9 ± 0.8</td>
<td>0.794</td>
</tr>
<tr>
<td>Donor cause of death (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVA</td>
<td>58</td>
<td>64</td>
<td>N/A</td>
</tr>
<tr>
<td>Head trauma</td>
<td>42</td>
<td>32</td>
<td>N/A</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>4</td>
<td>N/A</td>
</tr>
<tr>
<td>Brain death duration (days)</td>
<td>1.5 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>0.129</td>
</tr>
</tbody>
</table>

SEM, standard error of mean; BMI, body mass index; CVA, cerebrovascular accident
<sup>a</sup>Fisher’s exact test was used for this category variable
Fig. 1

Islet recovery rate post culture (%) vs. Duration of hypernatremia (hrs)

\[ y = -1.041 \times x + 82.63 \]

\( R^2 = 0.370 \)

\( p < 0.001 \)
Fig. 2

A

% beta cells

Normal control  |  Hypernatremia

B

% apoptotic beta cells

Normal control  |  Hypernatremia
Fig. 3

% successful graft function

<table>
<thead>
<tr>
<th></th>
<th>Normal control</th>
<th>Hyponatremia</th>
</tr>
</thead>
<tbody>
<tr>
<td>% successful</td>
<td>85%</td>
<td>42%</td>
</tr>
</tbody>
</table>

* indicates a significant difference.
Fig. 4

$P=0.022$
Fig. 5

AUC of BG (>200 mg/dl)

Normal control

Hyponatremia

*