Sodium levels of human pancreatic donors are a critical factor for determination of islet efficacy and survival

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Qi M, Valiente L, Bilbao S, Omori K, Rawson J, McFadden B, Juan J, Nair I, Mullen Y, El-Shahawy M, Dafoe D, Kandeel F, Al-Abdullah IH. Sodium levels of human pancreatic donors are a critical factor for determination of islet efficacy and survival. Am J Physiol Endocrinol Metab 308:E362–E369, 2015. First published November 23, 2014; doi:10.1152/ajpendo.00443.2014.—Organs from brain-dead donors were obtained from the human islet manufacturing database of the City of Hope. Donors with elevated hemoglobin A1c (Hb A1c ≥6.5%), DCD criteria, and cardiac downtime were all excluded from this analysis. Serum sodium levels were extracted from donor charts originally provided by organ procurement organization. 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 were categorized as the normal control group (n = 22), and those with serum sodium levels of ≥160 meq/l were considered to be 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.

THE ACUTE SHORTAGE OF DONOR PANCREATEA has increased the use of pancreata from marginal donors to meet the growing demand for clinical islet transplantation (20, 43, 46). Hence, it is important to evaluate the characteristics of donors that 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 (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 investigated thoroughly (14). Specifically, the association between donor serum sodium levels and islet isolation outcomes has yet to be analyzed.

Hypernatremia is commonly seen in brain injury patients (48, 50), especially those who develop central diabetes insipidus (DI) and/or overusage 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 lead to hypernatremia and increased plasma osmolality (30). Therefore, a sustained hypernatremia state in brain-dead donors eventually causes 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. (53) not only provided supporting evidence for the above results but also found that hypernatremia in donors corrected to 155 meq/l or less before procurement did not adversely impact liver graft survival. Furthermore, in the study by Totsuka et al. (53), deteriorated liver graft function after transplantation was explained by the fact that 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 postisolation. 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 (Hb A1c ≥6.5%), DCD criteria, and cardiac downtime were all excluded from this analysis. Serum sodium levels were extracted from donor charts originally provided by organ procurement organization. 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 were categorized as the normal control group (n = 22), and those with serum sodium levels of ≥160 meq/l were considered to be the hypernatremia group (n = 32).
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 by following the City of Hope 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 Technologies, Miami, FL). The distended pancreas was then cut into seven to 10 pieces and transferred to the Ricordi digestion chamber. After the pancreatic tissue was transferred, 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 (Sigma-Aldrich, St. Louis, MO) (32); once 50% of free islets were detected under the microscope, the digestion phase was stopped and the dilution phase 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 purification islet count from pooled digested tissue. Islet purification was done with Biocoll Separation Solution (Biechorn; Cedae Laboratory), using continuous density gradients (23). Purified islets were pooled based on purity into different fractions. Postpurification samples were subsequently taken from each fraction to acquire islet counts for each fraction, which were expressed as islet equivalents (IEQ) using previously described methods (44). Recovery rate of islets after purification was calculated based on following formula: recovery rate postpurification (% = (total IEQ no. postpurification/total IEQ prepurification) × 100. Isolated islets were then cultured in CMRL-1066 supplemented culture medium, as described previously (Mediatech, Manassas, VA) (19), at 37°C/5% CO2 for further assessment.

Islets were cultured for 24–72 h prior to assessment. Islet count and viability were determined using dithizone and fluorescein diacetate and propidium iodide (Sigma-Aldrich), respectively, following the previously described methods (4). The recovery rate postculture was calculated; recovery rate postculture (% = (total IEQ no. after culture/total IEQ postpurification) × 100. Glucose-stimulated insulin secretion (GSIS) was performed using static incubation or perfusion assay (5, 23). Assessments of cultured islets were conducted independently by a quality control team. Quantification of β- and apoptotic α-cells using laser scanning cytometry. Islets from hypernatremia (n = 20) and control (n = 20) groups pre- and postculture were processed for laser scanning cytometry (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 polyclonal guinea pig anti-insulin (DAKO cat. no. A0564) at 1:100 dilution. Secondary antibody was donkey anti-guinea pig Texas Red + Cy5 at 1:100 dilution. Apoptotic cells were assayed using a Takara kit (cat. no. MK500; Clontech Laboratories, Mountain View, CA) for detection of early apoptotic cells, using a modification of the method described by the manufacturer. The sections were stained for 10 min with DAPI (0.2 μg/ml; Sigma). The slides were scanned using an iCys laser scanning cytometer (ThorLabs, Newton, NJ), 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 β-cells was expressed by the percentage of insulin-positive cells in each preparation. Percentage of apoptotic β-cells was expressed by percentage of insulin and terminal deoxynucleotidyl transferase-mediated 2′-deoxyuridine 5′-triphosphate nick end labeling (TUNEL)-positive cells in each preparation (51). Islet transplantation in nonobese diabetic (severe combined immune deficiency) mice. All of 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 nonobese diabetic (severe combined immune deficiency) (NOD Scid) mice for islets isolated for clinical transplantation and research applications. The islet number (1,200 IEQ) transplanted per mouse in our center was established as an optimum islet mass. The culture periods (24–72 h) for the islets from two donor groups were similar. Typically, three NOD Scid mice (males, 10–12 wk of age) (The Jackson Laboratory, Bar Harbor, ME) 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) for 3 consecutive days. The mice with hyperglycemia (>350 mg/dl) for at least 2 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 two to three times/ wk for 30 days to measure blood glucose levels using a glucometer (LifeScan, Milpitas, CA). Successful graft function was achieved if two-thirds of transplanted mice were normoglycemic (<200 mg/dl) within 14 days and maintained normoglycemia levels for >20 days. Poor graft function was determined if the above criteria were not met. Statistical analysis. GraphPad Prism Software 6.0 (GraphPad, La Jolla, CA) was used to analyze the data and generate the figures. Unpaired Student’s t-test (2-tailed) or Fisher’s exact test was used to compare between the normal control and hypernatremia groups. Values were expressed as means ± SE. Linear regression analysis was used to determine the degree of correlation between the duration of hypernatremia and islet recovery rate postculture. 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)] vs. 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 of 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%), designated as the hypernatremia group, presented elevated serum sodium levels ≥160 meq/l, and the normal control group (n = 222) exhibited serum sodium levels of ≤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 (≥155 meq/l). However, the peak sodium level in the hypernatremia group (167.9 ± 0.9 meq/l) was significantly higher than that of the normal control group (152.8 ± 1.3 meq/l) (P < 0.001). As expected, the last records of serum sodium levels before procurement were increased significantly in the hypernatremia group (163.5 ± 0.6 meq/l) compared with the normal control group (145.9 ± 0.4 meq/l) (P < 0.001). No significant differences between hypernatremia and normal control groups were observed in terms of age, sex, or BMI. With regard 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 pancreas. Total IEQ prepurification, postpurification, and postculture were also similar between the two groups. Islet yields (IEQ/g of pancreatic tissue) postpurification and postculture also did not show significant differences. Furthermore, there were no significant differences when islet yields were analyzed by fractions. How-ever, the recovery rate of islets postculture was significantly lower in the hypernatremia group (59.1% compared with the normal control group (73.6%). In the hypernatremia group, the average hypernatremia duration was 23 h (range: 4–48 h). Duration of hypernatremia was inversely correlated with the islet recovery rate postculture (r² = 0.370, P < 0.001; Fig. 1). The recovery rate of islets during purification was not significantly different. Postculture islet viability and GSIS, the results showed lower glucose-stimulated insulin release in hypernatremia donor groups compared with the normal control group, although the differences were not significant.

LSC analysis showed that the percentage of β-cells in the hypernatremia group was 42.36 ± 2.51%, which was not significantly different compared with that in the normal group (44.88 ± 1.92%) (P = 0.426). Similarly, the percentage of apoptotic β-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 group (5 of 12 isolations, 42%) compared with the normal control group (45 of 53 isolations, 85%) (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: AUC = 0.697; 95% confidence interval: 0.538–0.857, P = 0.022 (Fig. 4). The optimal cutoff 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 >200 mg/dl during the 30-day followup was compared between the hypernatremia and normal control groups. The AUC for the hypernatremia group (3.443 ± 1.068) was significantly higher than that in the normal control group (1.083 ± 265, P = 0.019; Fig. 5), indicating diminished islet function in glycemic control as a result of hypernatremia.

Furthermore, the results of the ROC analysis showed that the graft function could be predicted by duration of hypernatremia (AUC = 0.896; 95% confidence interval: 0.682–0.110, P = 0.042; Fig. 6). The optimal cutoff points for duration of hypernatremia were >18.5 h (83% sensitivity and 100% specificity).

**DISCUSSION**

Brain-dead donors often develop severe DI, which has been linked to hypernatremia, particularly at the time of organ procurement (6, 47). Sodium levels greater than 145 meq/l are defined as hypernatremia in patients with severe traumatic brain injury (29). However, for patients with the potential to be donors for organ procurement for transplant application, hypernatremia of Na⁺ ≥160 meq/l negatively affects the 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 Na⁺ <160 meq/l would result in the exclusion of many donors for organ recovery. Therefore, in this study we chose sodium levels of 160 meq/l as the hypernatremia threshold. Hoefer et al. (21) hypothesized that donor hypernatremia may aggravate the existing reperfusion injury caused by intracellular sodium increase because of acidosis and H⁺/Na⁺ exchange during ischemia and cellular Ca²⁺ overload due to the Ca²⁺ influx. Dawwas et al. (11) and Gonzales et al. (17) reported that livers obtained from hypernatremic donors when

Table 3. Donor characteristics of 2 groups of islets that were randomly transplanted into diabetic NOD Scid mice

<table>
<thead>
<tr>
<th>Sodium Level</th>
<th>No. of donors</th>
<th>Donor serum sodium levels, meq/l</th>
<th>Admission sodium levels</th>
<th>Highest sodium levels</th>
<th>Last levels before procurement</th>
<th>Donor sex, %male/ female</th>
<th>BMI, kg/m²</th>
<th>Donor cause of death, %</th>
<th>Brain death duration, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥160 meq/l</td>
<td>12</td>
<td>140.8 ± 1.5</td>
<td>141.0 ± 0.6</td>
<td>165.8 ± 1.1</td>
<td>151.9 ± 0.8</td>
<td>163.0 ± 1.1</td>
<td>75/25</td>
<td>58</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>≤155 meq/l</td>
<td>53</td>
<td>141.0 ± 0.6</td>
<td>151.9 ± 0.8</td>
<td>145.5 ± 0.7</td>
<td>145.5 ± 0.7</td>
<td>145.5 ± 0.7</td>
<td>62/38</td>
<td>64</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>P Value</td>
<td></td>
<td>0.927</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.353</td>
<td>0.001</td>
<td>0.067*</td>
<td>0.794</td>
<td>0.129</td>
</tr>
</tbody>
</table>

Values are means ± SE. NOD Scid, nonobese diabetic (severe combined immune deficiency). *Fisher’s exact test was used for this category variable.
transplanted into recipients resulted in poor graft functions, suggesting that increased intracellular osmolality affects graft outcome. 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 in osmolality, and this in turn results in hepatocyte injury and liver obtained from donors with hypernatremia failing to function when transplanted; thus, correction of hypernatremia prior to procurement resulted in improved 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 (≥160 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 hypernatremic donors.

In this retrospective analysis, a total of 254 islet isolations were carried out, of which ~13% of the donors exhibited serum sodium levels ≥160 meq/l. Despite elevated sodium levels, differences in islet yield pre- and postpurification 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 postculture 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 blockers (ranolazine) inhibit glucagon release from pancreatic α-cells and reduce glucose levels in diabetic animals (13). In another study, Anazawa et al. (2) used chloride channel inhibitors during the rodent pancreatic digestion with collagenase for islet isolation, which significantly improved islet recovery and function. 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 postculture.

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-reduced T-type Ca2+ channel resulted in stimulation cytoprotective protein kinase pathway (40). Further-
more, it has also been reported that high salt concentration activates both the inflammatory pathway and the classic hypertonicity-induced p38 MAPK pathway (28). In our study using TUNEL assays, apoptotic β-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 the insulinoma cell line (HIT) is incubated in isotonic solution in the presence of the 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, result 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 finally, 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) at which, theoretically, ion channels are inactive. However, the digestion process with enzymes and mechanical dissociation to free the islets is carried out at 37°C, whereby the ion 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/extracellular osmolality (56), which in turn may cause cellular shrinking or swelling, affecting islets.

Previous studies have shown that the use of the antidiuretic 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 the anterior chamber of eye in live animals may unravel islet function and vascularization from hypernatremic donors (34, 49).

The biochemical profiles of donors have been shown recently to be important parameters to determine islet isolation outcomes as well (31). In particular, it has been reported recently that Hb A₁c has detrimental effects on isolation outcomes (12, 42). In this study, elevated Na⁺ at the time of pancreas procurement resulted in the decline of islet transplant outcomes in immune-deficient diabetic NOD Scid mice, especially those donors with >18.5 h of sustained hypernatremia and islet loss of >28% postculture. 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 preprocurement for this particular donor was 165 meq/l. Although the islet morphology was excellent, the islet recovery rate postculture was 52%, which was lower than the average in the hypernatremic group (59.1 ± 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 the success of clinical islet transplantation.

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

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


REFERENCES

Influence of hypernatremia and polyuria on swelling-induced insulin secretion.


