

MAGNETIC RESONANCE IMAGING AS NON-INVASIVE TOOL FOR PREDICTING
SUCCESS OF ISLET TRANSPLANTATION

BY

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ABSTRACT

The success of autologous islet transplantation greatly depends on the total islet yield. Using MRI as a non-invasive tool to determine the volume of the pancreas, the correlation between the calculated volume of the pancreas and the total islet yield has been determined. By using the volume of the pancreas as an indicator of total islet yield, MRI could be used as a predictive tool to determine the success of islet transplants. The data was gathered retrospectively from ten transplant patients, and the volume was determined by freehand contouring of the pancreas on MR images. With only ten patients, no significant correlation could be found between the volume vs. total islet yield, volume vs. IEQ (Islet Equivalent unit), mass vs. total islet yield, and mass vs. IEQ, with correlations of 0.238, 0.139, 0.343, and 0.219, respectively. The lack of correlation between the volume and total islet yield indicates other unaccounted variables such as fibrosis, inflammation, and fatty tissue infiltration that destroy islets but were not included in our volume calculations. Using a new protocol that takes the stated variables into account, we hope to find a better correlation between volume and total islet yield in future studies.

INTRODUCTION

Chronic Pancreatitis (CP), the inflammation of the pancreas that ultimately leads to permanent damage to the organ, is a disease that affects 3-9 persons in 100,000 (70% of which are alcohol-induced) [1]. The diseased pancreata from such patients present with high degree of fibrosis, inflammation, and fatty tissue infiltration. The final solution to the unremitting pain associated with CP is a pancreatectomy. However, by removing

the pancreas, the patient can no longer produce insulin or glucagon, thus resulting in surgically induced brittle diabetes [21]. Autologous Islet Transplant (AIT) has been a successful procedure for patients with total or near-total pancreatectomy [2][3][4][5][6][7][8][9]. The first AIT in a canine model was done in 1976 by Mirkovitch *et al.* [10]; the success from the procedure led to the first AIT in humans in 1977. Sutherland *et al* performed an AIT in a 39 years old female with familial pancreatitis [11][12]. It is important to note that the progress of islet transplantation techniques has led to allogeneic islet transplants for the treatment of type I diabetes. A patient with diabetes is able to use the islets isolated from a donor pancreas to produce endogenous insulin. Allogeneic islet transplantation provides improvements in glycemic control and decreases the risk of diabetic complications, and since only the islets of Langerhans are isolated and transplanted in the patient, it is a less invasive alternative to pancreas transplantation.

Although total pancreatectomy with AIT is offered as a last resort to CP patients, it offers many benefits. Relief from the intractable pain drastically improves the patient's quality of life [2][3][12][13][14], but the key benefit of AIT is to provide an endogenous source of insulin and glucagon in order to avoid brittle diabetes (Figure 1).

While there are tremendous benefits, the problems associated with AIT include diminishing function of transplanted islets over time [15][16][17][18], and insufficient number of transplanted islet [15][24]. To address the insufficient number of transplanted islets we aimed to use a non-invasive method of predicting the islet yield using MRI. Magnetic resonance imaging has gained much interest in islet transplantation in the recent years. The greatest focus of using MRI for islet transplantation has been on

tracking islets' viability and function post-transplantation. Jirak et al. was the first to show that islets could be detected in a rodent model after transplantation using Superparamagnetic Iron Oxide Nanoparticles [19]. Other methods of detection have also been explored. Barnett et al. used labeling agents in encapsulated islets [19] and Antkowiak et al. used Manganese-Enhanced MRI [19].

While the focus has been on post-operative use of MRI in islet transplantation, our goal is to use this non-invasive tool as means of predicting the islet yield from the volume of the pancreas, thus determining the success rate of the transplant. In 2003, a relationship between the volume of the pancreas achieved from CT data and the total islet yield was drawn [20]. Based on the correlation between pancreatic volume and total islet yield from findings in the previous work we hypothesized that the calculated volume of a pancreas based on MRI data can serve as an indicator of total islet yield.

MATERIALS AND METHODS

In this study, data from 10 previous patients were retrospectively gathered. Both male and females were represented with an average age of 42.7 ± 4.1 (age range of 26 to 64 years). These patients were diagnosed with CP and received total pancreatectomy with simultaneous AIT. The procedures were completed at the University of Arizona Medical Center between June 2011 and February 2012.

Volume Calculation via MRI

Two of the patients' pancreatic volume was determined by the ECHO (Spoiled Gradient Echo) sequence while all others were determined using LAVA (Ultra Fast

Gradient Echo) sequence. In order to determine the volume, the pancreas was traced using free hand contouring as seen in Figure 3. The accuracy of the outlining was confirmed by an experienced radiologist. Since each slice included some overlap with the previous slice, the following formula was used to calculate the volume using the area and the depth of each slice:

$$V_{Pancreas} = (Average\ Area\ of\ Adjacent\ slices + 1st\ slice + last\ slice) * \frac{1}{2} slice\ thickness$$

Autologous Islet Transplantation Procedure

The AIT procedure begins simultaneously with total pancreatectomy. Once the pancreas is excised, the duodenum and any excess retroperitoneal fat is removed. The pancreatic duct is cannulated and transported to the isolation lab for processing. The pancreas is weighed and gross observations are noted. In the more recent procedures, the volume of the pancreas is also determined using a graduated cylinder via displacement (data not shown). The excised pancreas is then extended with a Collagenase solution (Collagenase HA, Vitacyte Indianapolis, Indiana). Islets are liberated from the surrounding exocrine tissue by mechanical and enzymatic dissociation using an automated method, as previously described [22][23]. In brief, the pancreatic tissue is placed in a digestion chamber with a recirculating solution containing collagenase at 37°C in order to sustain optimal digestion. The degree of digestion was continually assessed by staining with dithizone to determine the optimal timing to discontinue the digestion process. Once the digestion is complete, the enzymatic reaction is halted by cooling the islet containing solution to 7-10°C and diluting the solution with culture medium. Before transplantation, islet preparations were re-suspended in 25% human

serum albumin with 70 IU/kg of heparin. Three 100-uL aliquots are removed from the re-suspended tissue, added to a Petri dish, stained with dithizone and manually counted. Recovered islets were transported into the operating room and infused into the liver through the portal vein. Portal vein pressure was closely monitored during the infusion to avoid portal hypertension and potential portal vein thrombosis. The protocol is summarized in Figure 2, while a more detailed protocol can be found in Rodriguez-Rilo et al [21]. A specimen of the the pancreas was also sent to pathology to determine the degree of fibrosis.

Statistical Analysis:

Statistical Analysis was performed using Microsoft Excel. Correlation was calculated using the calculated volume, mass, total islet yield, and Islet Equivalent units (IEQ)

RESULTS

Volume Calculation

Due to the nature of this study, the source of calculating the volume of the pancreata slightly differed in some patients. The total islet yield was compared to the calculated volume and the mass of the pancreas. The IEQ was also compared to the volume and the mass of the pancreas. The IEQ is the islet equivalent unit based on an average islet diameter of 150 μm . Lastly, the mass of the pancreas was compared to its volume. The time lapse between the MRI and the AIT ranged between 10-748 days. With the exception of two patients, the MRI was within 100 days of the surgery (Table 1).

The mass and volume of the pancreas ranged between 25.61g-129.99g (mean =72.28g), and 21.52mL-110.15mL (mean = 53.54mL), respectively (Table 1). The mass of the pancreas and its volume were found to have a significant correlation with a value of $r=0.868$ and $p=0.0005$ (Figure 4). The total islet yield and IEQ had a range of 27000-816000 (mean = 256.353), and 5910-437000 (mean= 179947), respectively (Table 1). The comparison of pancreatic mass and total islet yield produced a correlation of 0.343 and $p=0.3024$ (Figure 5). The relationship of mass and IEQ was also found to be not significant with a correlation of 0.219 and $p=0.5176$ (Figure 6). The volume of the pancreas was not significantly correlated to the total islet yield or IEQ with correlation values of 0.238 and 0.139 and p-values of 0.4814 and 0.6832, respectively (Figures 7 & 8).

Finally, the degree of fibrosis was determined using a scoring system of 1-6. In this system, fibrosis is described as 1) focal, weak 2) focal, moderate 3) focal, strong 4) diffuse, weak 5) diffuse, moderate 6) diffuse, strong. Not all pancreata were analyzed; however, the five that were analyzed received a score of 4 or higher (Table 1).

DISCUSSION:

CP is a disabling disease that significantly alters the well being and the quality of life of patients suffering from it. Despite the remarkable progress in the field of pathophysiology of chronic pancreatitis, the treatment of the disease remains a challenging entity. Treatment options for patients with chronic pancreatitis are extremely limited. Initial treatment, nearly always conservative, includes the use of nonopioid analgesics, followed by opioids. A total pancreatectomy is usually the last resort,

performed only after medical management and endoscopic, interventional, or surgical drainage procedures have been exhausted; however, it is associated with the development of severe “brittle diabetes” as well as exocrine insufficiency. AIT simultaneous with total pancreatectomy is an accepted approach used to alleviate pain and to provide a certain degree of metabolic stability in a select series of patients with chronic pancreatitis in whom other medical and standard surgical approaches have failed. The success of this procedure heavily depends on the number of islets isolated from the diseased pancreas. While there are many factors that affect the outcomes of the total islet yield, this study has focused on the volume of the pancreas as a determinant of the total islet yield. Using MRI data from ten patients to determine the volume of the pancreas, it appears that the correlation between the volume and the total islet yield is weak. The same could be said about the correlation between the mass vs. total islet yield, volume vs. IEQ, and mass vs. IEQ.

The weak correlation could be due to the small number of patients included in the study. With only ten data points, the results could be skewed and not be representing the true nature of the relationships. Human error in outlining the pancreas in MR images is another variable. With no available computer program to calculate the volume or outline the organ, we were left with free hand contouring of the pancreas. Determining the volume of the organ during the surgery through displacement could mitigate this issue. Therefore, since the beginning of this study, the volume displacement has been determined during the surgery. However, for those patients who received the operation previous to our study, there are no gross volumes to compare to the deduced volumes.

The most probable reason for lack of correlation is the fact that these pancreata are diseased at the time of transplantation. Chronic Pancreatitis causes fibrosis, inflammation, and fatty tissue infiltration into the pancreas, which causes the destruction of insulin producing cells. While the pancreas can be viewed and outlined on an MRI, it is difficult to determine the degree of fibrosis, inflammation, and fatty tissue infiltration. Thus the volume calculated from the MRI is not an accurate representation of the islet producing portions of the pancreas. At this junction, we propose the development of new techniques such as those developed in Dr. Altbach's group to get a more complete image of the pancreas. Using advanced MRI techniques, Dr. Altbach is working on developing a "quality" factor for the pancreas (QFP). The QFP will be derived from: (1) the percentage of fatty tissue infiltration obtained from a fat-water imaging sequence (FWMRI), (2) the apparent diffusion coefficient of water (ADC) determined from diffusion-weighted MRI (DWMRI), and (3) the spin-spin relaxation time (T2). The ADC and T2 values will be used individually or combined as a biomarker of inflammation and fibrosis.

The data from the QFP can be integrated with the volume data in order to provide a more complete picture of the usable parts of the pancreas. Using such protocol will provide an accurate and non-invasive method of obtaining the volume, which can be correlated to the total islet yield and thus be used as a predictive tool for AIT.

REFERENCES

1. Kocher HM, Kadaba R. *Chronic pancreatitis*. Clin Evid (Online). 2011 Dec 21;2011. pii: 0417.
2. Farney, A.C., et al., *Autotransplantation of dispersed pancreatic islet tissue combined with total or near-total pancreatectomy for treatment of chronic pancreatitis*. Surgery, 1991. **110**(2): p. 427-37; discussion 437-9.
3. Robertson, R.P., et al., *Prevention of diabetes for up to 13 years by autoislet transplantation after pancreatectomy for chronic pancreatitis*. Diabetes, 2001. **50**(1): p. 47-50.
4. Frey CF, B.B., *Surgery in Chronic Pancreatitis*. Clinics in Gastroenterology, 1984(13): p. 913-940.
5. Prinz, R.A., *Surgical options in chronic pancreatitis*. Int J Pancreatol, 1993. **14**: p. 97-105.
6. Morrison, C.P., Wemyss-Holden S.A., Partensky C, Maddern G.J., *Surgical Management of intractable pain in chronic pancreatitis: past and present*. J Hepatobiliary Pancreat Surg, 2002. **9**: p. 675-682.
7. Mc Cullagh, E., J.R. Cook, and E.K. Shirey, *Diabetes following total pancreatectomy; clinical observations of ten cases*. Diabetes, 1958. **7**(4): p. 298-307.
8. Dresler, C.M., et al., *Metabolic consequences of (regional) total pancreatectomy*. Ann Surg, 1991. **214**(2): p. 131-40.

9. Gruessner, R.W., et al., *Transplant options for patients undergoing total pancreatectomy for chronic pancreatitis*. J Am Coll Surg, 2004. **198**(4): p. 559-67; discussion 568-9.
10. Mirkovitch, V. and M. Campiche, Successful intrasplenic autotransplantation of pancreatic tissue in totally pancreatectomised dogs. Transplantation, 1976. **21**(3): p. 265-9.
11. Sutherland DER, M.A., Najarian JS, *Pancreatic Islet Cell Transplantation*. Surg Clin North Am, 1978. **58**: p. 365-382.
12. Wahoff, D.C., et al., *Autologous Islet Transplantation to Prevent Diabetes After Pancreatic Resection*. Ann Surg, 1995. **222**:(4): p. 562-579.
13. Clayton, H.A., et al., *Pancreatectomy with islet autotransplantation for the treatment of severe chronic pancreatitis: the first 40 patients at the leicester general hospital*. Transplantation, 2003. **76**(1): p. 92-8. Rodriguez Rilo, H.L., et al., *Total pancreatectomy and autologous islet cell transplantation as a means to treat severe chronic pancreatitis*. J Gastrointest Surg, 2003. **7**(8): p. 978-89.
14. Ahmad SA, L.A., Wray CJ, D'Alessio D, Choe KA, James LE, Gelrud A, Matthews JB, Rilo HLR, *Factors Predicting Success Following Islet Auto-Transplantation in 45 Patients with Severe Chronic Pancreatitis*. JACS, 2005. **In Press**.
15. Bellin MD, Sutherland DE, Beilman GJ, et al. Similar islet function in islet allotransplant and autotransplant recipients, despite lower islet mass in autotransplants. Transplantation; 91(3): p. 367-72.

16. Maedler, K., et al., *Glucose-induced beta cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets*. J Clin Invest, 2002. **110**(6): p. 851-60.
17. Robertson, R.P., et al., *Glucose toxicity in beta-cells: type 2 diabetes, good radicals gone bad, and the glutathione connection*. Diabetes, 2003. **52**(3): p. 581-7.
18. Robertson, R.P., et al., *Beta-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes*. Diabetes, 2004. **53 Suppl 1**: p. S119-24.
19. Borot S, Crowe LA, Toso C, Vallée JP, Berney T, *Noninvasive imaging techniques in islet transplantation*. Curr Diab Rep, 2011 Oct; 11(5): 375-83.
20. Choe KA AS, Lowy AM, Somogyi L, Soldano DA, Pennington LJ, Matthews JB, Rilo HL. *CT findings in the liver following total pancreatectomy and autologous islet cell transplantation*. in *Radiological Society of North America's 90th Scientific Assembly and Annual Meeting*. 2004. Chicago, Illinois.
21. Rodriguez Rilo HL, Ahmad SA, D'Alessio D, et al. Total pancreatectomy and autologous islet cell transplantation as a means to treat severe chronic pancreatitis. J Gastrointest Surg 2003; 7(8): p. 978-89.
22. Ricordi C, T.A., Carroll PB, et al., *Human islet isolation and allotransplantation in 22 consecutive cases*. Transplant, 1992. **53**: p. 404-414.
23. Fontes, P.A., et al., *Human islet isolation and transplantation in chronic pancreatitis using the automated method*. Transplant Proc, 1992. **24**(6): p. 2809.
24. Morrison, C.P., et al., *Islet yield remains a problem in islet autotransplantation*. Arch Surg, 2002. **137**(1): p. 80-3.

TABLES

Patient ID	Volume (mL)	Mass (g)	Total Islet Yield	IEQ	Fibrosis Score	Duration Between MRI & AIT
AZAIT0015	21.52	25.61	234000	437000	6	35
AZAIT0017	38.87	47.17	27000	5910	5	26
AZAIT0018	56.19	80.9	816000	106620	4	57
AZAIT0022	47.60	57.04	339000	250830	5	99
AZAIT0023	30.20	54.04	63000	41100	NA	748
AZAIT0024	52.85	112.1	288000	299000	NA	113
AZAIT0025	38.74	44.77	105333	56867	6	58
AZAIT0027	72.05	98.88	348000	242700	NA	71
AZAIT0028	110.15	129.99	289200	289200	NA	29
AZAIT0029	67.23	72.27	54000	70240	NA	10

TABLE 1

FIGURE LEGENDS

Figure 1: A) The difficult choice that chronic pancreatitis patients face before a pancreatectomy operation. While suffering from pain, the surgery could result in surgically induced brittle diabetes. B) The solution to this problem by implementing a autologous islet transplantation. This allows the body to naturally produce insulin to avoid diabetes while terminating the pain from the pancreas.

Figure 2: A summary of the steps in an AIT. It begins from the pancreatectomy, to islet isolation, and finally transplanting the islets into the portal vein of the patient.

Figure 3: A) The outlining of the pancreas can be seen from this MRI using the LAVA protocol. The freehand contouring of each slice ultimately provides the volume. B) The ECHO protocol was also used in some patients to find the volume. While each protocol serves a different purpose in diagnosis, for volume calculating purposes the protocol with the clearest images were used.

Figure 4: Pancreatic mass vs. calculated volume shows the close relationship of the two variables with a correlation of 0.868.

Figure 5: The relationship of pancreatic mass vs. total islet yield with a correlation of 0.343.

Figure 6: The relationship of pancreatic mass vs. islet equivalent unit with a correlation of 0.219.

Figure 7: The relationship of calculated volume vs. total islet yield with a correlation of 0.238.

Figure 8: The relationship of the calculated volume vs. islet equivalent unit with a correlation of 0.139.

FIGURES

**The Difficult Choice Presented
with Chronic Pancreatitis**



FIGURE 1 (A)

**Islet Replacement is a
Potential Solution for this Problem**

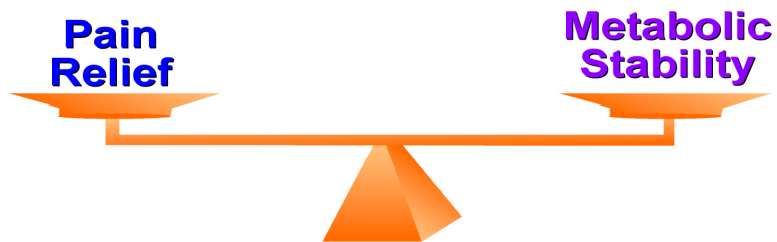


FIGURE 1 (B)

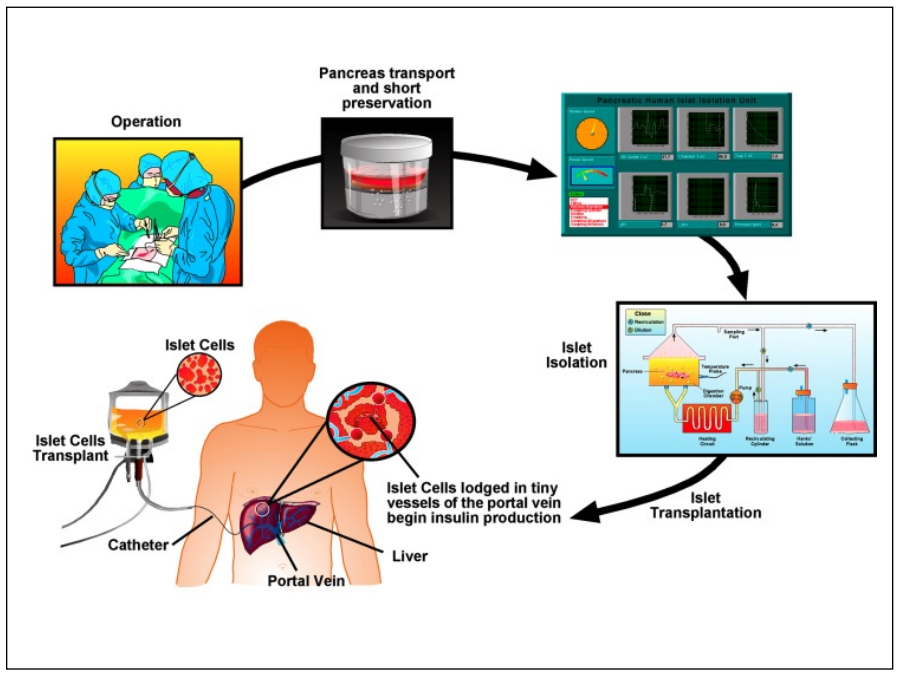


FIGURE 2

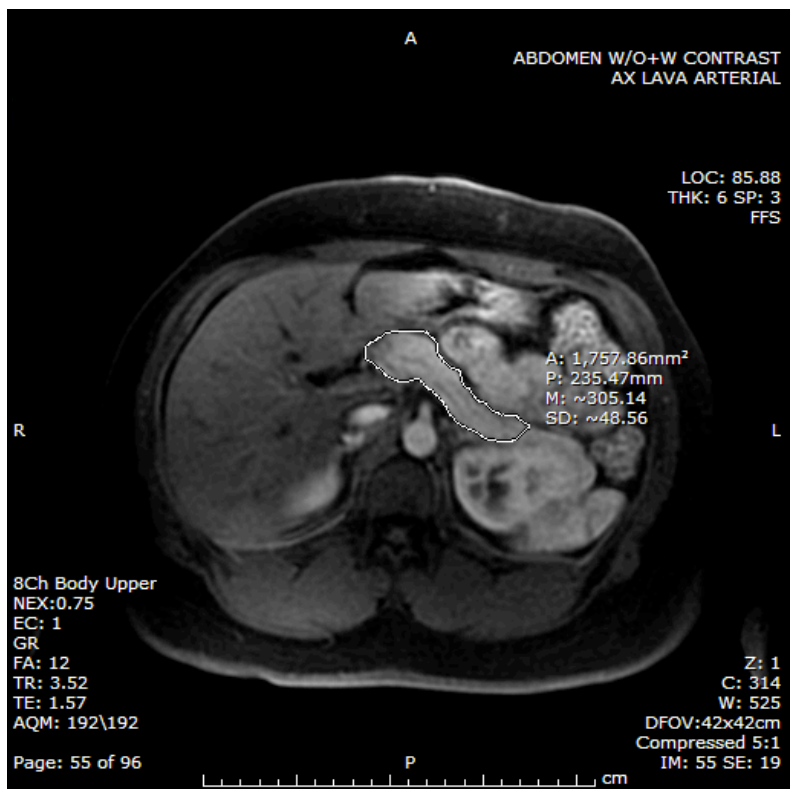


FIGURE 3 (A)



FIGURE 3 (B)

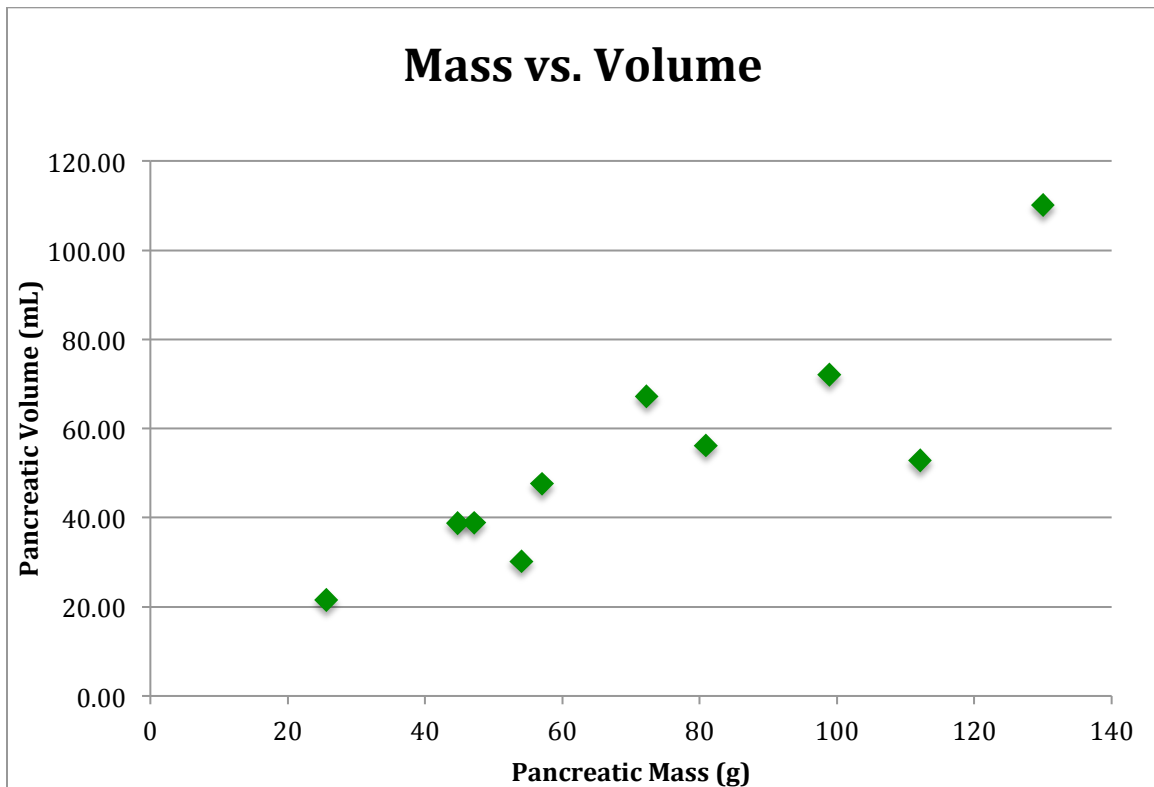


FIGURE 4

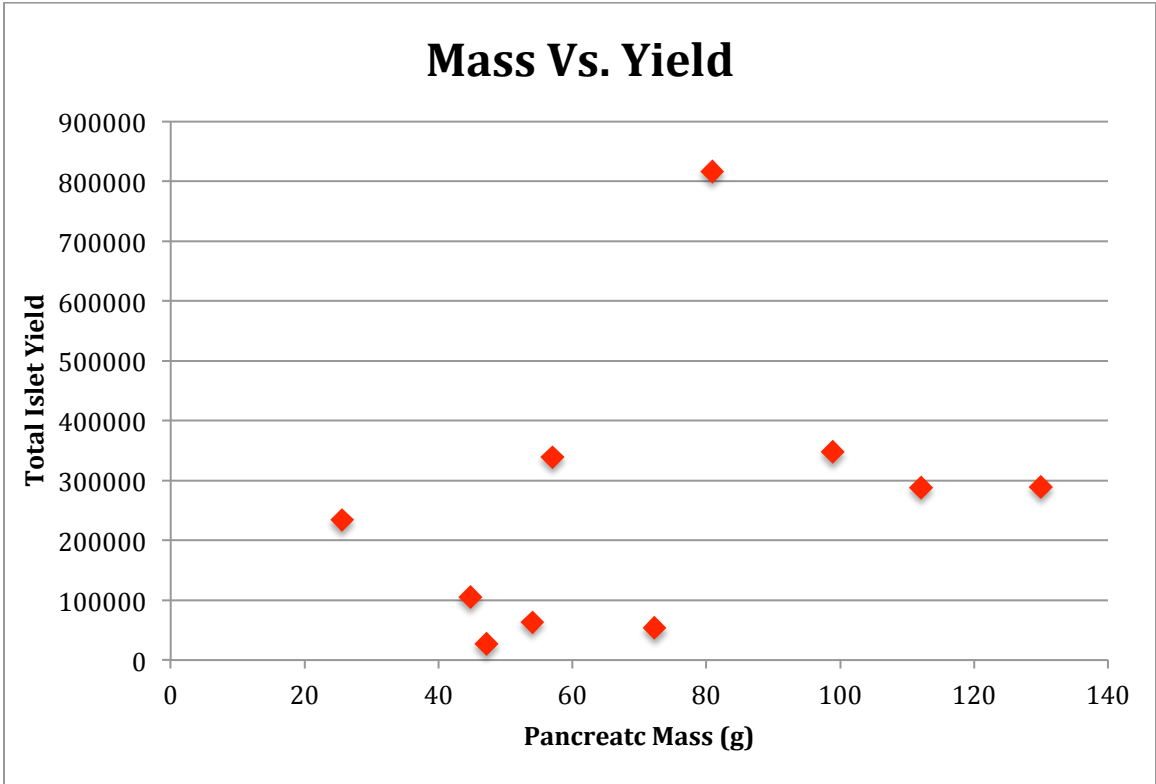


FIGURE 5

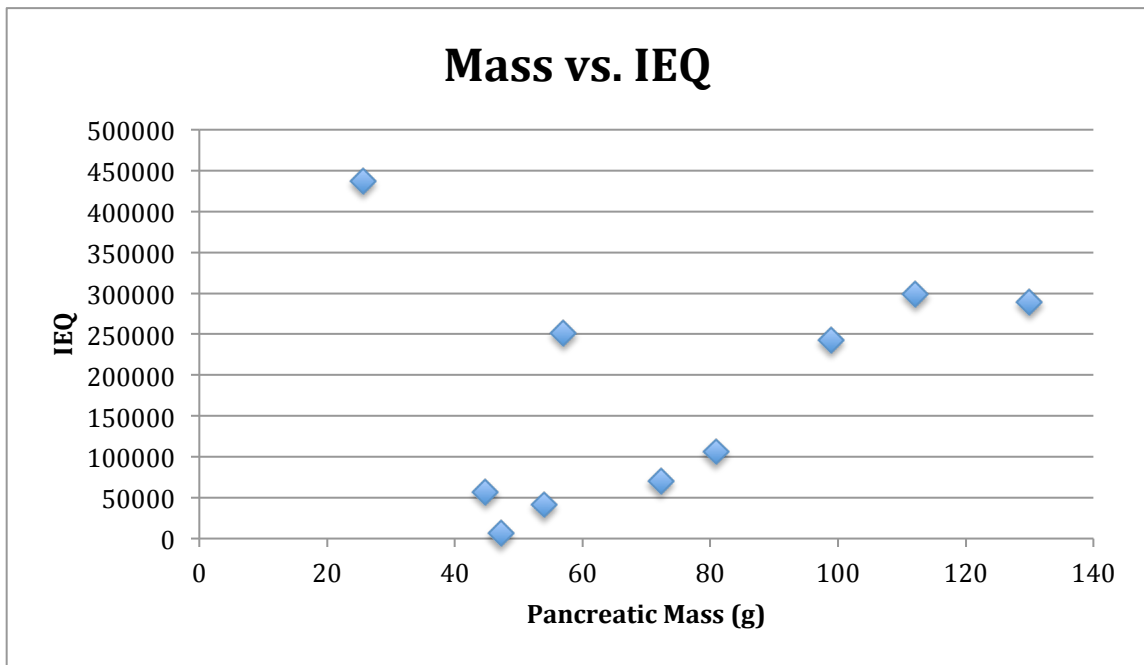


FIGURE 6

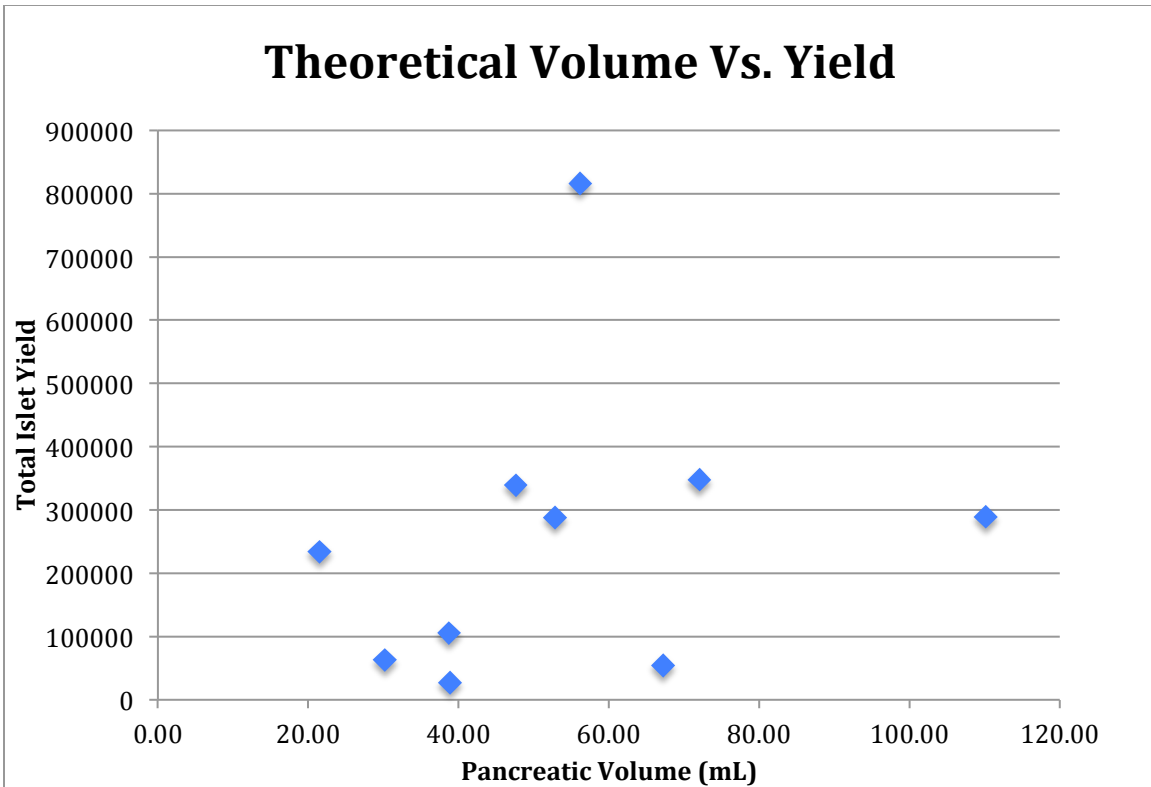


FIGURE 7

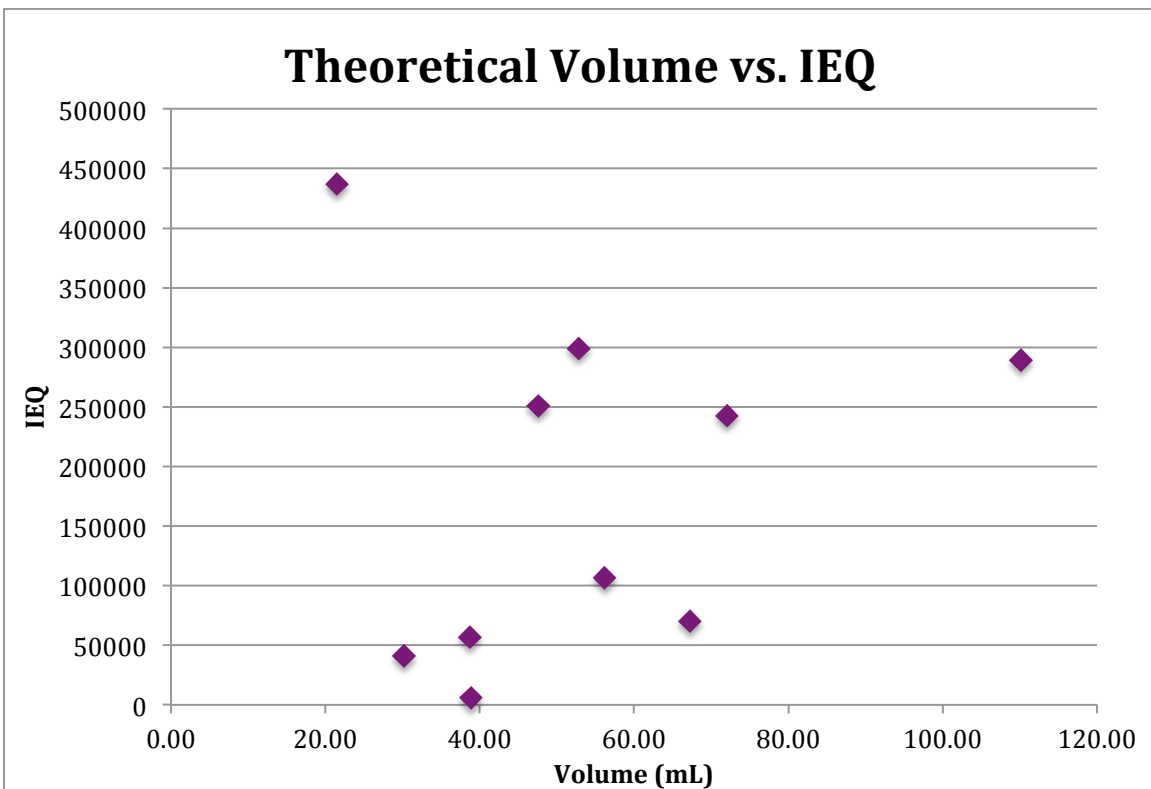


FIGURE 8