ORIGINAL ARTICLE METASTATIC PANCREATIC CANCER

Progression to Fragmentation during Cellular Isolation Improves Clinical Glycemic Outcomes in Patients Undergoing Islet Cell Transplantation

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ABSTRACT

Context Islet autotransplantation (IAT) involves a complex islet isolation process in which the explanted pancreas is mechanically and enzymatically digested to separate islet cells from exocrine pancreatic tissue. Islet cells transition during the isolation process from being "embedded" in exocrine pancreatic tissue, to becoming "free" once separated from the exocrine tissue, to eventually becoming "fragmented" with ongoing digestion. However, it is unclear if the relative percentage of "embedded", "free" or "fragmented" islet cells affects subsequent pancreatic endocrine function. **Objective** Evaluate the effect of each type of islet cell on endocrine function in patients who underwent IAT. **Design** Retrospective cohort study. **Setting** Academic tertiary medical center **Patients** 33 patients who underwent IAT from 2015-2020. **Main Outcome Measures** Percent change in pre-operative *vs.* three month post-operative c-peptide levels. **Results** There was a weak correlation between the percent change in c-peptide and number of "fragments" (R^2=0.0156) and percent of islet cells 'embedded" (R^2=0.0011). However, as the number of "fragments" increased and percent "embedded" decreased, the percent change in C-peptide approached zero. Genetic etiologies (CFTR, SPINK1, PRSS1, CTCR, and CPA1) resulted in lower percent decreases in c-peptide than non-genetic etiologies (9% vs. 52%, p=0.152) although this trend did not reach statistical significance. **Conclusion** Although a weak correlation existed between the number of "fragments" and the percent change in c-peptide, the data support erring on the side of allowing the digestion to proceed to more "fragments" and fewer "embedded" islets in order to enhance glycemic outcomes. As such, we suggest stopping digestion when no more "embedded" islets are observed.

INTRODUCTION

Islet autotransplantation (IAT) is a novel procedure for treating chronic pancreatitis (CP) and/or recurrent acute pancreatitis (RAP), debilitating diseases that often causes pancreatic dysfunction and life-limiting pain [1, 2]. IAT can reduce the risk of developing type 3c-diabetes associated with pancreatectomy [3, 4]. The islet isolation process is complex, involving mechanical and enzymatic digestion of the explanted pancreas [5]. During the isolation process, the islets progress from being "embedded" in pancreatic exocrine tissue to being separated from the tissue ("free"). With further digestion, the "free" islets subsequently become degenerated into "fragments".

During the isolation process, the isolationist must decide when to stop the digestion as the process progresses from "embedded" to "fragments." Stopping the digestion too early might lead to more "embedded" islets; stopping too late may lead to more islet "fragments." Currently no standard protocol for when to stop the digestion exists, resulting in different stopping points based on the isolationist's subjective discretion [6]. It also remains unclear whether the relative percentage of "embedded" *vs.* "fragments" influences the subsequent pancreatic endocrine function when these cells engraft in the liver.

A clearer idea of how the relative number of each type of islet cells impacts subsequent pancreatic endocrine function would enhance the efficacy of IAT and thus further reduce the risk of developing type 3c-diabetes. This study therefore sought to evaluate the effect of each type of islet cell—"embedded," "free," and "fragments"—on pancreatic endocrine function in patients who underwent IAT. *A priori*, we hypothesized that allowing the isolation process to proceed relatively further toward the development

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of "fragments" would lead to improvement in clinical glycemic outcomes.

METHODS

Patient Selection

This investigation was a retrospective cohort study approved by the Dartmouth Committee for the Protection of Human Subjects. The study included 33 patients who underwent IAT at Dartmouth-Hitchcock Medical Center (DHMC) from 2015-2020. All patients had the pancreatectomy and the islet cell isolation performed intraoperatively at DHMC. Included patients had preoperative, intraoperative, and post-operative data available.

IAT Procedure

The IAT procedure begins with surgical excision of the pancreas using an open or laparoscopic technique. In order to minimize ischemia and maximize islet cell perfusion, the splenic and gastroduodenal blood vessels are preserved. As a result, arterial inflow and venous outflow are maintained until just prior to removal. The explanted pancreas is then placed in an ice-cold, antibiotic, static preservation solution (SPS-1) bath [5].

After the pancreas is explanted, the organ undergoes isolation as follows. The gastroduodenal artery in the head and the splenic artery in the tail are cannulated with a webster cannulae and flushed with the either Wisconsin solution or Ringer's lactate until venous outflow is clear. Non-pancreatic tissue including lymphovascular tissue is then trimmed from the specimen bluntly. A 16 gauge angiocath is then inserted into the main pancreatic duct in the head and body and secured with silk ties. Then, a warm enzyme solution containing proteases, collagenases, and buffers (Vitacyte LLC, Indianapolis IN) is infused into the parenchyma of the pancreas with manual pressure using a 60 cc syringe is repeatedly injected into the pancreas via the angiocath. Using scissors, the pancreas is mechanically fractionated into approximately 5 mm pieces.

These pieces are then placed into a Ricordi digestion chamber. The chamber is shaken and warmed to 37 degrees Celcius to assist chemical digestion. Every 3-5 minutes, samples are collected and stained with dithizone for inspection under a microscope to examine islet number, size, and form. The stopping point for digestion is subjective, but once the digestion is finished, the chamber is cooled to 4 degrees Celsius. The digested cells are gathered, combined with 5% human serum albumin to stop enzyme digestion, and then centrifuged. The islets are washed by re-suspending the cells with Hank's balanced salt solution (first wash contains 2% with the second containing 20% Hank's solution). The final collected cells are suspended with 5% human serum albumin and 35 U/kg of heparin [5]. Of note, COBE purification is not performed at our center.

Once the islets are ready to be transplanted, the patient receives 35 U/kg of intravenous (IV) heparin to mitigate the risk of portal vein thrombosis. A 16-gauge needle with attached IV tubing is then used to infuse the islets directly into the portal venous system. Portal pressures are monitored during the transplantation to limit the risk of thrombosis [7].

Data Collection

A database containing the patients' demographics, procedural details, and lab results was created and organized and collected via chart review. The primary outcome was the percent change in pre-operative *vs*. three month post-operative c-peptide levels. C-peptide was selected as the measure of glycemic control, as smaller percent changes in c-peptide indicate consistent insulin production and thus better glycemic control. C-peptide also remains independent of exogenous insulin use.

STATISTICS

We evaluated continuous variables using the student's t-test and categorical variables using the Fisher Exact test. Linear regression was also utilized to determine the correlation between two variables. We performed the statistical analysis using Microsoft Excel (Microsoft Corporation, Redmond, WA). Any analysis involving "fragments" excluded the one patient without data for total number of "fragments" (yielding n=32 subjects), whereas any analysis involving c-peptide included this patient (resulting in n=33 subjects). The p-value for statistical significance was <0.05.

RESULTS

Patient Characteristics

We identified 42 patients who had undergone islet cell transplant at our institution since 2015. Those without post-operative 3-month c-peptide were excluded (n=9). Therefore, 33 patients in total were analyzed. One of the 33 did not have data for the total number of "fragments", so this patient was excluded from the analysis involving number of "fragments". Baseline patient characteristics are shown in Table 1. Of the 33 patients included in the study, 11 were male and 22 were female. 25 received total pancreatectomies, while eight underwent either completion (n=3) or partial (n=5) pancreatectomy. Of patients with genetic etiologies (n=18), the gene responsible was CFTR in 38.9% of patients, PRSS1 in 16.7%, SPINK1 in 16.7%, SPINK1/CFTR in 11.1%, CPA1 in 5.6%, CTCR in 5.6%, and associated with another etiology in 5.6%.

Clinical Outcomes

The quality control group is displayed in Figure 1, which graphs the number of "fragments" by the percent of islets "embedded." During the islet cell isolation process, as more "fragments" are produced, the number of "embedded" cells decreases.

Our primary outcome is shown in Figure 2a. There was a weak correlation between percent change in c-peptide and number of "fragments" (R^2=0.0156) and between percent

Table 1. Baseline patient characteristics

	(N=33)
Baseline	
Male, n (%)	11 (33)
Female, n (%)	22 (67)
Age – years (SD)	46 (14)
Operative	
Total, n (%)	25 (76)
Completion, n (%)	3 (9)
Partial, n (%)	5 (15)
Etiology	
Alcohol, n (%)	5 (15)
Triglyceride, n (%)	2 (6)
Idiopathic, n (%)	4 (12)
Pancreas Divisum, n (%)	1 (3)
Central Pancreatectomy, n (%)	1 (3)
Gallstone, n (%)	2 (6)
Genetic, n (%)	18 (55)
PRSS1, n	3
SPINK1, n	3
CPA1, n	1
CFTR, n	7
SPINK1/CFTR, n	2
CTCR	1
N/A	1

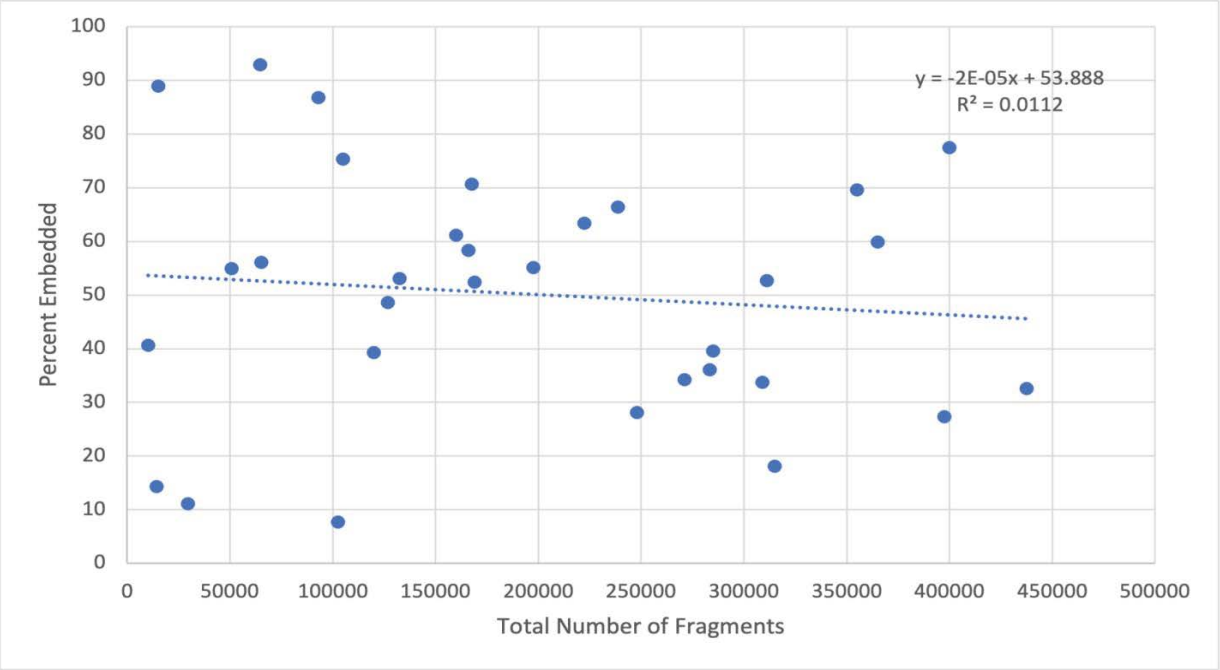

Figure 1. Quality control group. Percent of islets embedded by total number of fragments.

change in c-peptide and percent of islet cells "embedded" (R^2=0.0011). However, Figure 2b demonstrates that as the number of "fragments" increased and percent "embedded" decreased, the percent change in c-peptide approached zero.

Of the 33 patients analyzed, the mean BMI was 25.83 kg/m^2 ± 5.66 kg/m^2. Figure 3a graphs mean percent decrease in c-peptide by BMI, which was grouped according to standard deviations above and below the mean. Figure 3a demonstrates that patients with healthier BMIs experienced a lower percent decrease in c-peptide levels than those with high BMIs (greater than 31 kg/m^2) or low BMIs (less than 20 kg/m^2), though this value did not reach statistical significance (46% for healthy *vs*. 66% for unhealthy, p=0.225).

Figure 3b displays the mean percent change in c-peptide according to age grouped by standard deviations above and below the mean age of 46.09 years ± 14.01 years. Patients older than 60 years experienced statistically significant worse glycemic control than those younger than 60 years (mean percent change in c-peptide of -77% *vs*. -4%, p=0.012).

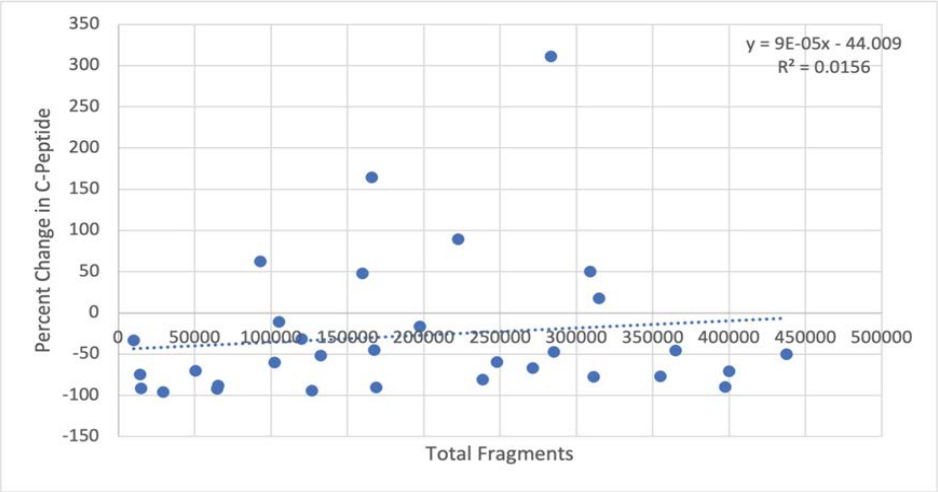

Figure 2a. Primary Outcome-Mean percent change in c-peptide as a function of total fragments.

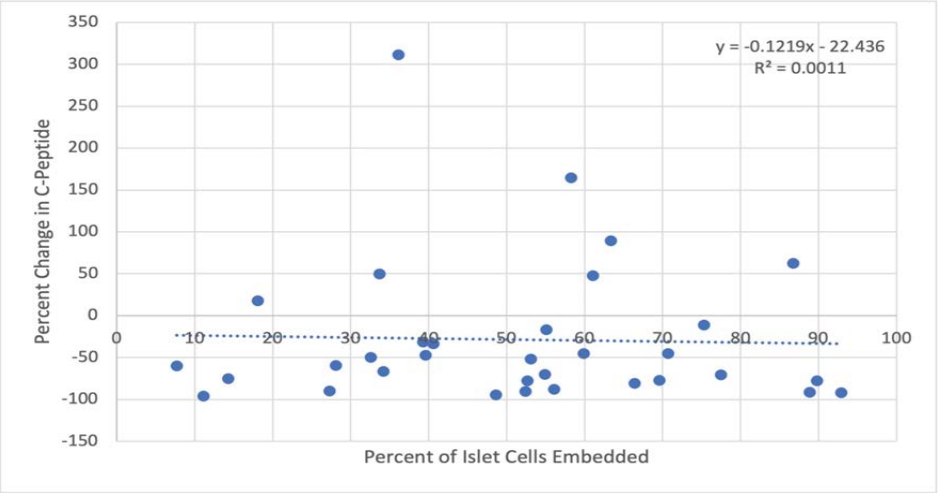

Figure 2b. Primary Outcome- Mean percent change in c-peptide as a function of percent of islets embedded.

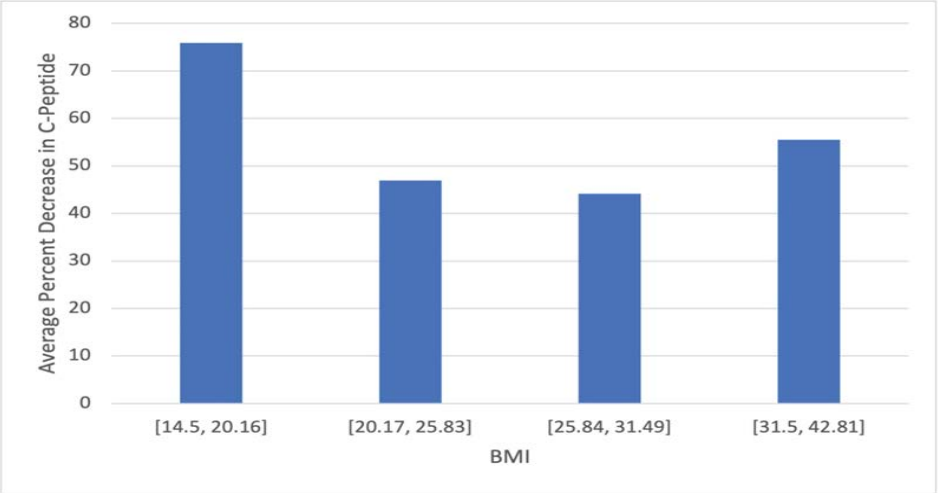

Figure 3a. Mean percent decrease in c-peptide by BMI.

Mean percent decrease in c-peptide levels according to gender is shown in Figure 3c. Females experienced a significantly lower percent decrease in c-peptide compared to males (12% *vs*. 62%, p=0.037).

Although not reaching statistical significance, the mean percent decrease in c-peptide among patients with genetic etiologies for CP was smaller than the mean percent decrease in c-peptide among patients with non-genetic etiologies (9% *vs*. 52%, p=0.152), as demonstrated by Figure 3d.

There was no statistically significant difference between the mean percent change in c-peptide in patients receiving completions compared to the mean percent change in c-peptide in patients receiving TPIAT, but

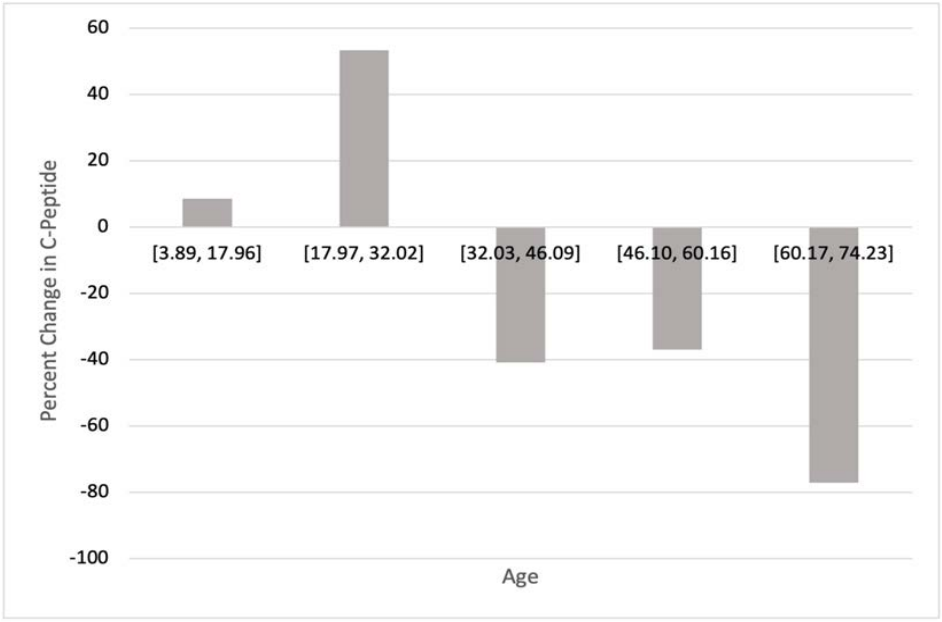

Figure 3b. Mean percent change in c-peptide by age.

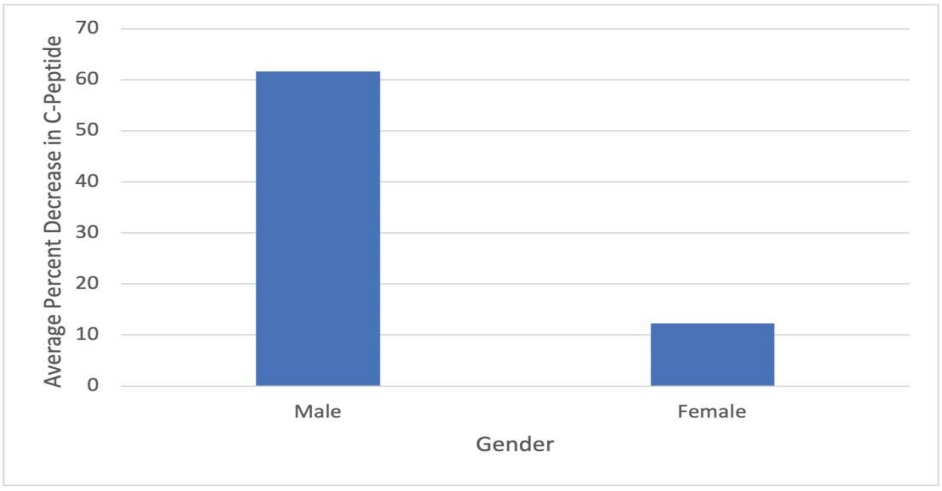

Figure 3c. Mean percent decrease in c-peptide by gender.

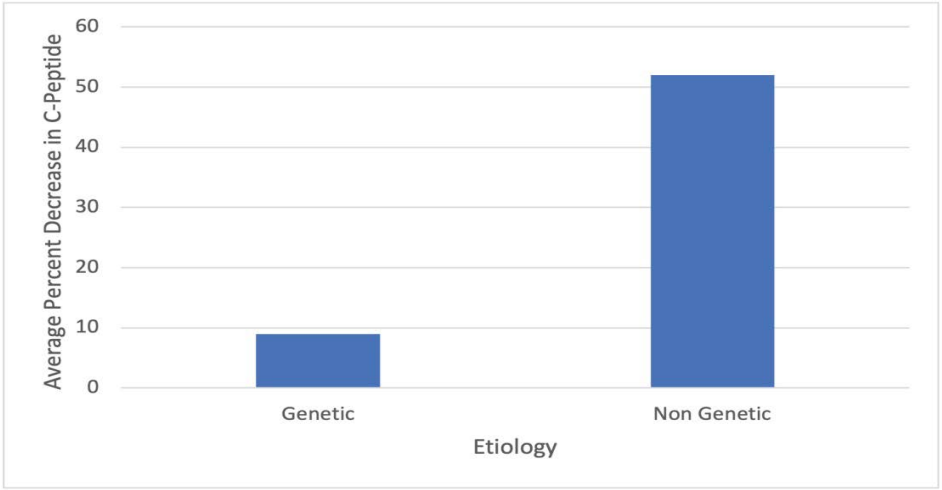

Figure 3d. Mean percent decrease in c-peptide by etiology.

Figure 3e demonstrates that those undergoing completion pancreatectomy did experience a larger mean percent decrease in c-peptide than those receiving totals (48% *vs.* 33%, p=0.713).

As shown by Figure 3f, patients with CT scans displaying evidence of CP had greater percent decreases in c-peptide than those with CT scans that did not display evidence of CP (50% *vs.* 3%, p=0.149), though this value did not reach statistical significance.

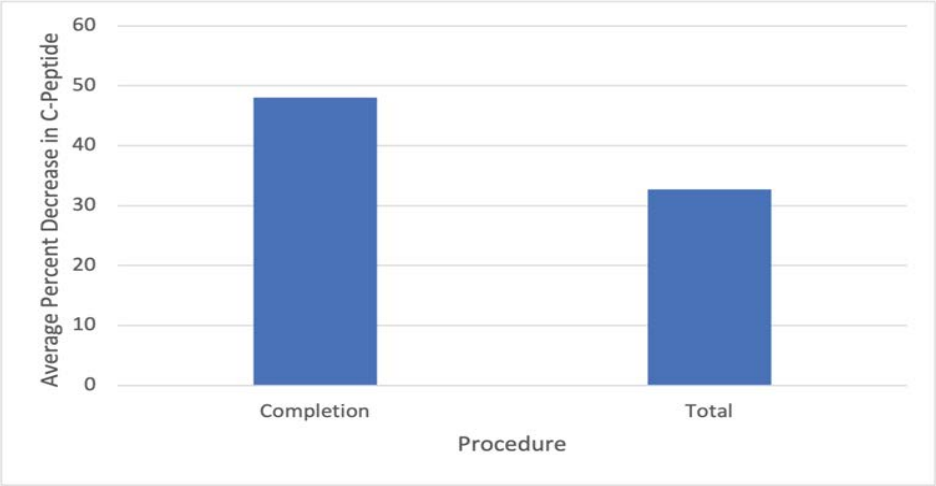

Figure 3e. Mean percent decrease in c-peptide by procedure.

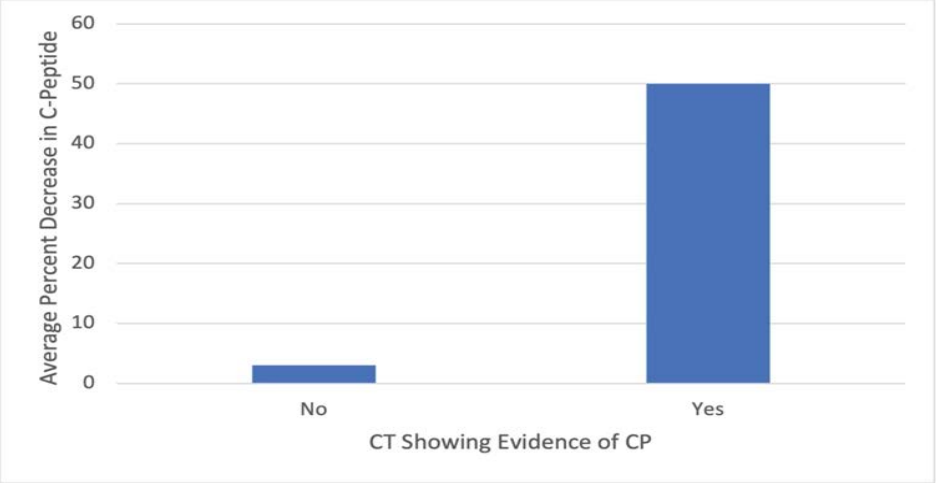

Figure 3f. Mean percent decrease in c-peptide by CT showing evidence of CP.

DISCUSSION

This retrospective study revealed that as pancreatic digestion proceeded, thereby yielding more "fragments" and fewer "embedded" islet cells, the percent change in c-peptide approached zero, which is indicative of better glycemic control. As such, in order to maximize glycemic outcomes, isolationists who do not perform COBE purification should err on the side of allowing further digestion toward "fragments". In fact, based on these data we suggest stopping isolation once that are no further embedded islets observed.

Islet cell transplantation was first used in 1977 as a way to mitigate the effects of developing type 3c diabetes in patients who had undergone pancreatectomies [8, 9]. Since then, the improvement of isolation techniques has fashioned IAT into a pragmatic approach for treating CP and RAP [3, 10]. Even though the isolation process has been refined since 1977, currently no standard protocol exists for when to stop the digestion of the pancreas and harvest the islets, which leaves the stopping point up to the subjective discretion of the isolationist. A clearer idea of when to stop the digestion would enhance the outcome of islet transplantation to generate better glycemic control in patients undergoing IAT. Improved technique results in increased islet yield, and thus a greater chance for the patient to remain insulin independent post-operatively. In addition, improved patient selection from our findings may also increase the efficacy of the procedure.

Local isolation with dedicated islet isolation facilities represents the IAT gold standard [3]. Most IAT procedures at these institutions begin with open removal of the pancreas followed by a similar digestion process described earlier involving chemical digestion with proteases and collagenases, mechanical digestion with a Ricordi chamber, and resuspension in human serum albumin. Many of these centers also perform a COBE purification step in which the islets are further separated from the pancreatic exocrine tissue [11]. COBE purification takes advantage of the different densities of the islets and of the exocrine tissue. Despite IAT's promising results in treating CP and RAP, the procedure remains uncommon due to the need for specialized islet isolation equipment and training. Such isolation labs are expensive and consequently few of them exist.

As a solution to the scarcity of dedicated islet isolation facilities, pancreatic surgical centers without such labs have collaborated with institutions containing these facilities in order to make IAT more accessible to this patient population [6]. In remote isolation, the resected pancreas is transported to a remote institution where the isolation occurs, and then the separated islets are transported back and injected into the patient. Although local isolation results in better islet function following transfusion (likely due to reduced ischemia time in local isolation), remote isolation results in similar rates of insulin independence as local isolation, which makes remote isolation a functional alternative when access to a local isolation facility is not feasible [6, 12, 13].

From 2012 to 2015, our center (Dartmouth-Hitchcock Medical Center) performed remote IAT using Massachusetts General Hospital's islet isolation facility. In 2015, we began performing intraoperative islet isolation (local islet isolation without a dedicated islet isolation lab) in a method first reported by Fan et al. [14]. After the surgical removal of the pancreas, islet isolation occurs in the operating room. Once the islets are digested, centrifuged, isolated, and combined with heparin and human serum albumin (all performed in the same operating room), they are infused into the portal venous system for engraftment into the liver. We do not perform COBE purification. Our center has found that intraoperative isolation is comparable to remote isolation with regard to IEQ/kg [15]. Furthermore, insulin requirements, mean c-peptide levels, and hemoglobin A1c are comparable between the intraoperative isolation and the remote isolation [15]. Given these findings demonstrating islet graft function, in addition to lower hospitalization and procedural costs, intraoperative isolation is effective and should increase the access to IAT [15].

Efforts to refine this intraoperative isolation process could make an even greater impact and further improve the efficacy of this treatment. A better understanding of the mechanics of the isolation process comes from the results in this study, as Figure 1 indicates the number of "fragments" increases as the percent of "embedded" cells decreases. Although not a perfect correlation, the data do show a progression from "embedded" to "free" to "fragments". This finding confirms quality control expectations that there is a progression in the isolation process. As digestion proceeds, the islets become increasingly separated from exocrine tissue, which leads to the production of more "fragments".

Although our study found a weak correlation between the number of "fragments" and the percent change in c-peptide, the data support erring on the side of allowing the digestion to proceed to more "fragments" and fewer "embedded" islets in order to achieve enhanced subsequent endocrine function. More "fragments" and fewer "embedded" islets result in better glycemic control because less attached exocrine tissue allows for better engrafted islet function. Less exocrine tissue likely results in less inflammation and less oxidative stress, which engenders better islet engraftment [4]? More "fragments" also lead to transplantation of less tissue foreign to the liver which consequently may improve islet engraftment.

One weakness of the study is that we could not perform multivariable regression analysis give the lack of data points to evaluate which factor had the most effect of glycemic control – *i.e.* type of islet, BMI, gene involvement, etc. However, given that the type of islets harvested during isolation is the one modifiable factor on glycemic control, we do believe that the message of the study is important.

Given our findings, our center will now allow the digestion to continue longer than previously, as erring on the side of more "fragments" and fewer "embedded" improves glycemic outcomes. Although a direct correlation does not exist between number of "fragments" and percent change in c-peptide, having more "fragments" in the isolation does appear to enhance subsequent endocrine function in patients undergoing IAT. As a result, we will use this study to guide our decision-making to halt the digestion in our isolation processes when "embedded" islets are no longer observed.

In addition, our findings will likely influence our patient selection for IAT. Young, healthy females with less severe CP seem to have the best clinical outcomes following IAT. Our results are consistent with a study performed by Ahmad et al. demonstrating patients with a BMI greater than 28 kg/m^2 resulted in worse glycemic control [11, 16]. Our results are also consistent with other studies that have demonstrated the greater likelihood of females to achieve insulin independence when compared to males [11]. Younger patients experience significantly better endocrine function following IAT compared to elderly, likely due to greater health, physicality, and plasticity. Similarly, patients without evidence of CP on cross-sectional imaging display less severe CP and thus experienced better outcomes following IAT than those with such evidence. Completions are also preferred to partials because less interference with the pancreas could preserve more islets.

Although we believe this study indicates longer digestion periods result in increased efficacy of IAT for improved islet functioning, additional research is needed to confirm these findings and to determine the relative percentages of "fragmented" islets versus "embedded" islets that lead to the most effective islet transplantation and functioning. Our study is limited in that our primary outcome was only available at three months post-operatively (percent change in three-month c-peptide). Future studies could investigate longer-term percent changes in c-peptide, as it is known that insulin independence decreases over time [17]. We ultimately decided not to examine percent change in HbA1c in addition to percent change in c-peptide since HbA1c levels are influenced by exogenous insulin. Furthermore, procedural variation could affect the results of the study. For our data analysis, we included partial pancreatectomies and completion pancreatectomies instead of just total pancreatectomies. We also do not

perform the extra COBE purification step, so this study does not reveal how using COBE purification affects our findings. Regardless of any shortcomings, the study offers important insight into refining islet isolation.

CONCLUSION

In conclusion, this study has demonstrated that erring on the side of producing more "fragments" during the islet isolation process results in better endocrine functioning post IAT. As such, we suggest stopping digestion when no more "embedded" islets are observed.

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