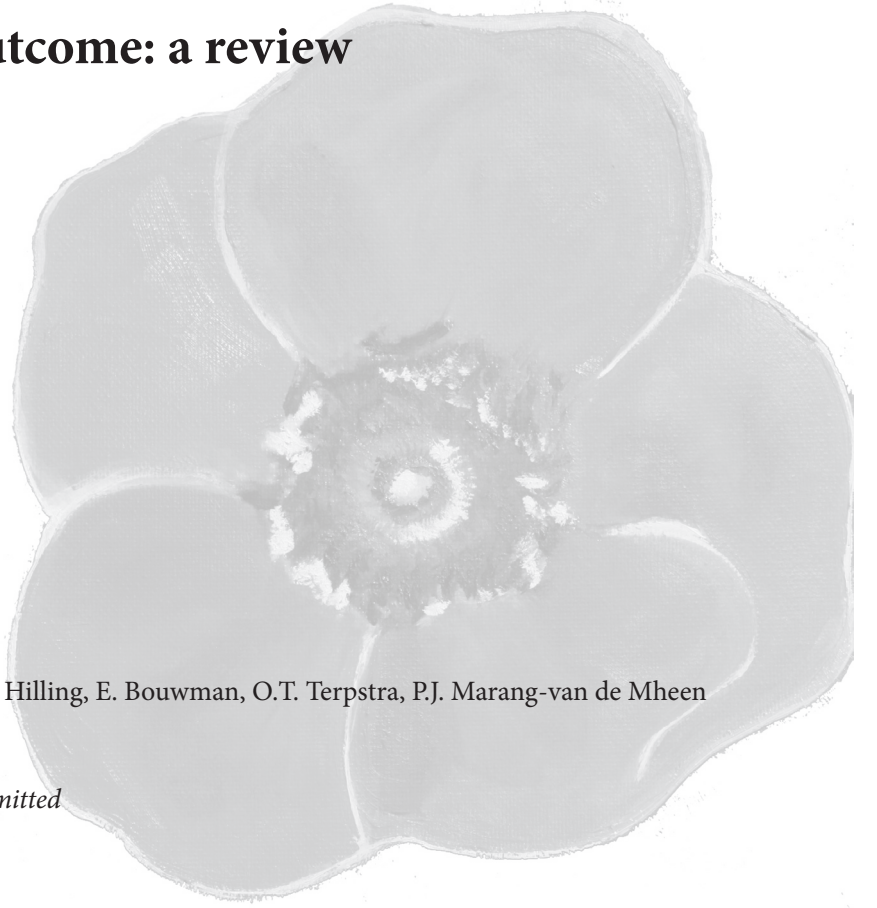
# Part II

## Human islet transplantation

# Chapter 4

## Effects of donor, pancreas and isolation-related variables on human islet isolation outcome: a review



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## ABSTRACT

### Introduction

Different factors have been reported to influence islet isolation outcome, but vary between studies and are hampered by small study samples per study. The purpose of this study was to perform a systematic review to assess the impact of donor, pancreas and isolation-related variables on successful human islet isolation outcome.

### Methods and Materials

Pubmed, Embase and Web of Science were searched electronically in April 2009. All studies reporting on donor, pancreas and isolation-related factors relating to pre-purification, post-purification islet isolation yield and proportion of successful islet isolations were selected. 74 retrospective studies had sufficient data and were included in the analyses.

### Results

Higher pre-and post-purification islets yields and a higher proportion of successful islet isolations were obtained when pancreata were preserved with TLM, rather than UW in donors with shorter cold ischemia times (one hour longer cold ischemia time resulted in an average decline of pre-purification, post-purification yields and proportion of successful isolations of 59IEQ/g, 54 IEQ/g and 21%, respectively). Higher pre-purification yields and higher percentage successful islet isolations were found in younger donors with higher BMI. Lower yields were found in donation after brain death (DBD donors) compared to donation after cardiac death (DCD donors). Higher post-purification yields were found for isolation with Serva collagenase.

### Conclusion

This review identified donor, pancreas and isolation-related factors that influence islet isolation yield. Standardized reports of these factors in all future studies may improve the power, identify additional factors and thereby contribute to improving islets isolation yield.

## INTRODUCTION

Transplantation of islets of Langerhans can improve metabolic control and quality of life in patients with longstanding type 1 diabetes. Despite improvement and standardization of isolation procedures, the outcome of human islet isolation remains unpredictable and highly variable. Furthermore, generally more than one islet preparation is required per recipient to achieve insulin independence after transplantation (1-6).

Previous studies have reported donor and other factors associated with higher success rates in terms of attaining adequate islet numbers for transplantation (7-13). However, different factors have been identified and large-scale trials in humans demonstrating the influence of a set of donor factors are lacking. Because previous studies are relatively small, factors could be missed. Therefore, different factors could be identified when studying larger numbers of donors and the question remains which factors independently affect islet isolation outcome when corrected for the effect of other variables.

Because there is a shortage of donor pancreata relative to the needs of potential transplant recipients, optimal use of the available donor organs is vital. We carried out a systematic review of the literature on human studies reporting on donor, pancreas and isolation-related factors and their influence on isolation outcome. In this way we can identify factors that have an independent effect on islet isolation outcome.

## METHODS AND MATERIALS

### Study selection

PubMed, Embase and Web of Science were searched to retrieve articles in English on human islet isolation from 1966 onwards.

The following search string was used:
("Islets of Langerhans Transplantation"[Mesh] OR (("Islets of Langerhans"[Mesh] OR "islets"[all fields]) AND ("transplantation"[MeSH Terms] OR "transplantation"[All Fields])))
AND
("isolation"[all fields] OR "Cell Separation"[Mesh] OR "Separation"[all fields] OR "Tissue and Organ Harvesting"[Mesh] OR "Harvesting"[all fields] OR "Tissue and Organ Procurement"[Mesh] OR "Organ Preservation Solutions"[Mesh] OR "Solution"[all fields] OR "Solutions"[all fields] OR "tissue donors"[MeSH Terms] OR "donor"[All Fields] OR "donors"[All Fields])
AND

(yield[All Fields] OR yields[All Fields] OR "isolation outcome"[All Fields] OR "isolation outcomes"[All Fields] OR "isolation result"[All Fields] OR "isolation results"[All Fields] OR harvest[All Fields] OR profit[All Fields] OR profits[All Fields] OR earnings[All Fields] OR earning[All Fields] OR output[All Fields] OR "success rate"[All Fields] OR "success rates"[All Fields] OR "recovery"[All Fields])

The search resulted (by April 2009) in 412 Pubmed, 60 Embase and 228 Web of Science titles, constituting a total of 702 titles. Two independent reviewers (DEH and PJMvdM) examined titles and read relevant abstracts to decide if the full-text articles should be obtained. Cases of disagreement were resolved by discussing the title and abstract. Full-text articles (n = 141) were examined and selected based on the following criteria: (1) Reporting on either donor, pancreas or isolation-related variables and their relation to islet isolation outcome, (2) Reporting isolation outcome in IE/g pancreas pre- or post-purification (3) sufficient specification of "successful" and "unsuccessful" islet isolation outcome as used in that study, (4) sufficient specification of donor organs used for islet isolation procedures with respect to selection characteristics.

Exclusion criteria were: (1) Histologically obtained pancreas variables and their relation to islet isolation outcome (2) Animal donor, pancreas and isolation-related variables and their relation to islet isolation outcome

Literature references were checked to minimize the risk of missing relevant studies. For duplicate papers reporting on the same study, we selected the article that reported the most complete and detailed data. This resulted in a total of 74 studies, eligible for further analysis (7-12, 14-81).

**Data extraction**

Data were extracted independently by DEH and PJMvdM by means of a predefined form. The following topics were included based on data availability in at least 50% of the studies:

General variables: year of index admission, country of study, number of pancreata in the study

- Donor pancreas variables: age, body mass index (BMI), last serum glucose before procurement, donation after brain death (DBD donors)/donation after cardiac death (DCD donors)
- Pancreas variables: pancreas weight, cold ischemia time (CIT), method of preservation
- Isolation variables: method of purification (continuous vs discontinuous and Ficoll vs other), brand of collagenase
- Study results: islet isolation outcomes in terms of pre-purification isolation yield, post-purification isolation yield, proportion of successful islet isolations (according to the definitions in the particular study).

**Statistical analysis**

Since the number of pancreata varied considerably between studies we weighted all isolation outcomes by the number of pancreata per study in all analyses. We studied the previously listed variables with respect to their relation with 3 outcomes: pre-purification isolation yield, post-purification isolation yield, and proportion of successful islet isolations.

We first performed univariate analysis, relating each variable to each of the 3 outcomes. However, since the effect of some factors on isolation outcome may be confounded by others, a multivariate analysis was performed, including only the variables that had a significant effect on isolation outcome in the univariate analysis. In this way, the independent effect of each of the variables on the 3 outcomes was assessed. The analysis with the outcome proportion of successful islet isolations was adjusted for differences between studies in the criteria used to define successful by including the criterium as a variable in the multivariate analysis.

## RESULTS

A total of 74 studies met our inclusion criteria, all retrospective studies. When studies compared different groups in relation to isolation outcome (e.g. TLM vs UW), these were included as separate groups, giving a total of 132 groups that were finally compared in the analysis.

When studies addressed both pre- and post-purification isolation yield and/or proportion of successful isolations, we included the studies in the analyses of each outcome.

**Pre-purification isolation yield**

Thirty-nine studies (7, 9-12, 14, 20, 21, 27, 31, 32, 34, 37-45, 48-57, 59, 62-64, 71, 75, 76, 80), 70 groups in total, reported characteristics influencing pre-purification isolation yield. Univariate analysis showed several factors to significantly affect pre-purification isolation outcome (Table 1): higher yields were obtained in studies with younger donors, with higher BMI, without a last glucose or a low last glucose reported, with relatively few DBD donors, short cold ischemia time and preservation with TLM rather than UW. These effects remained in multivariate analysis (Table 2), suggesting that each of these factors independently influenced pre-purification yield. For example, from donors who are one year older, on average a 64IEQ/g lower pre-purification yield was obtained. Furthermore, when cold ischemia time was 1 hour longer, on average a 59IE/g lower pre-purification yield was obtained, independently from other factors.

Less than 50% of the included studies reported data on pancreas weight and isolation specific characteristics so these were excluded from the analysis.

**Post-purification isolation yield**

Fifty-nine studies (7, 9-12, 14-20, 23-27, 30, 31, 33-37, 39-47, 50-56, 59-63, 65-70, 72-79, 81), 106 groups in total, reported characteristics related to post-purification isolation yield. Univariate analysis showed several factors to significantly affect post-purification isolation outcome (Table 1): higher yields were obtained in studies without a last glucose or a low last glucose reported, with relatively few DBD donors, short cold ischemia time, preservation with TLM rather than UW, purification with Ficoll and isolation with Serva collagenase. In multivariate analysis (Table 2), these effects remained as independent significant effects influencing post-purification isolation yield, except for last glucose before procurement and purifcation with Ficoll. For example, when cold ischemia time was 1 hour longer, on average a 54IEQ/g lower post-purification yield was obtained, independently from other factors.

In contrast with pre-purification yield, age, BMI and last glucose before procurement are no independent predictors of post-purification yield.

Less than 50% of the included studies reported data on pancreas weight and isolation specific characteristics so these were excluded from the analysis.

**Proportion of successful isolations**

Thirty-one studies (7-12, 22, 23, 28-30, 32, 34, 36, 38, 44, 48, 51, 52, 57, 58, 60, 66, 68, 69, 73-75, 78-80), 57 groups in total, reported characteristics related to the proportion of successful isolations. In univariate analysis (Table 1) higher yields were obtained in studies with younger donors, with higher BMI, without a last glucose or a low last glucose reported, with relatively few DBD donors, short cold ischemia time, higher pancreas weight and preservation with TLM rather than UW. In multivariate analysis (Table 2) these effects remained as independent predictors of a high percentage of successful isolations, except that higher percentage successful isolations were found in studies that did reported the last glucose before procurement. Furthermore, the percentage DBD donors had no independent significant influence on the percentage of successful islet isolations.

For example, from donors who are one year older on average a 1% lower percentage of successful isolations was obtained. Furthermore, when cold ischemia time was 1 hour longer, on average a 21% percentage of successful isolations was obtained, independently from other factors.

In contrast with pre-purification yield, percentage DBD donors is not and pancreas weight is an independent predictor as well as age, BMI and last glucose before procurement in contrast with post-purification yield.

In total, data of 2198, 4122 and 2769 pancreata were available for uni- and multivariate analysis of pre- and post-purification yield and proportion of successful islet isolations, respectively. However, in univariate analysis, 12.5% to 33.7% of the pancreata were

excluded in at least 1 analysis due to missing data. In multivariate analysis this was even higher (79.4-89.7%) since studies had to report on all of the variables included in the analysis, to have their pancreata included.

## DISCUSSION

The present study has shown that donor, pancreas and isolation-related factors have an influence on both pre- and post-purification islet isolation outcome, as well as on proportion of successful islet isolations.

Higher islets yields and a higher proportion of successful islet isolations were obtained when pancreata were preserved with TLM, compared to UW. This is in accordance with Agrawal et al (13). In their meta-analysis, significantly higher yields were found in pancreata preserved with TLM compared to UW. However, in their study, they found an equal rate of successful islet isolations in both groups. A possible explanation for this difference with our study could lie in the fact that in our multivariate analysis, the influence of TLM is corrected by other factors that have an influence on islet isolation yield.

Higher BMI and shorter cold ischemia times were also associated with higher islet isolation outcome pre- and post-purification as well as with a higher percentage of successful isolations when looking at cold ischemia time. Since larger islets are usually encountered in patients with higher BMI to obtain the higher insulin demand and longer cold ischemia times result in more damage to the islets, these results seem to have face validity and have been well reported in previous studies (11, 12, 19, 52, 58, 60, 82).

Our study showed lower isolations yields and proportion of successful isolations in studies with a higher percentage of DBD donors. This is remarkable since in previous studies, generally, higher yields were found in DBD donors compared to DCD donors. However, successful islet isolations from DCD donors have also been reported previously (3, 48, 81, 83). In our multivariate analysis, studies with a large percentage of DBD donors had significantly lower yields in pre-, post-purification isolation outcome and also a lower proportion of successful isolations, when adjusted for the effects of other variables. Part of the explanation could be that in previous studies there was insufficient power to correct for other variables. In the studies that did correct for other factors, the results could be prone to the effect of the other, potentially underreported, factors in the models. This last explanation could also have an effect on our results as well. Furthermore, results of different studies can not be easily compared without correcting for certain factors like age, since an age difference of 1 year has an influence on pre-purification islet yield of 64IEQ/g.

This study is a first attempt to look at the effect of donor, pancreas and isolation-related factors on isolation outcome. When reports of these variables in future

**Table 1.** Factors influencing pre- and post-purification isolation yield and proportion of successful islet iolations

Univariate analysis

| | Pre-purification isolation yield | | Post-purification isolation yield | | Proportion of successful islet isolations | |
|---|---|---|---|---|---|---|
| | B | 95% CI | B | 95% CI | B | 95% CI |
| **Donor** | | | | | | |
| Age (yrs) | -74.83 | -85.97; -63.68 | NS | | -1.08 | -1.36; -0.81 |
| Body Mass Index (kg/m2) | 59.78 | 45.03; 74.53 | NS | | 1.84 | 1.44; 2.24 |
| Last serum glucose before procurement (mmol/l) | -13.14 | -18.10; -8.19 | -2.70 | -4.29; -1.11 | -0.10 | -0.19; -0.02 |
| Last serum glucose before procurement reported (yes/no) | -509.35 | -630.48; -388.22 | -173.10 | -279.78; -66.43 | -4.70 | -8.02; -1.39 |
| Percentage DBD donors | -24.32 | -28.12; -20.53 | -15.59 | -20.82; -10.36 | -0.38 | -0.54; -0.23 |
| **Pancreas** | | | | | | |
| Cold ischemia time (hours) | -46.83 | -66.67; -26.99 | -72.27 | -89.69; -54.84 | -1.63 | -2.35; -0.91 |
| Pancreas weight (g) | * | | * | | 1.23 | 1.08; 1.39 |
| Preservation fluid (TLM vs UW) | 713.83 | 544.23; 883.43 | 596.84 | 463.11; 730.58 | 14.82 | 8.59; 21.05 |
| **Isolation** | | | | | | |
| Brand of collagenase (Serva vs other) | * | | 1688.03 | 1529.14; 1846.92 | * | |
| Brand of collagenase (Sigma vs other) | * | | -1122.67 | -1253.74; -991.58 | * | |
| Purification (continuous vs discontinuous) | * | | NS | | * | |
| Purification (Ficoll vs other) | * | | 104.74 | 4.93; 204.55 | * | |

* Data available in less than 50% of the studies

**Table 2.** Factors influencing pre- and post-purification isolation yield and proportion of successful islet iolations

Multivariate analysis

| | Pre-purification isolation yield | | Post-purification isolation yield | | Proportion of successful islet isolations | |
|---|---|---|---|---|---|---|
| | B | 95% CI | B | 95% CI | B | 95% CI |
| **Donor** | | | | | | |
| Age (yrs) | -64.07 | -86.50; -41.64 | * | | -1.04 | -1.22; -0.87 |
| Body Mass Index (kg/m2) | 151.62 | 110.63; 192.61 | * | | 8.02 | 7.26; 8.77 |
| Last serum glucose before procurement reported (yes/no) | -1929.34 | -2180.30; -1678.38 | NS | | 57.61 | 55.66; 59.55 |
| Percentage DBD donors | -32.35 | -37.93; -26.78 | -11.31 | -15.37; -7.25 | Constant in model | |
| **Pancreas** | | | | | | |
| Cold ischemia time (hours) | -58.74 | -94.37; -23.11 | -54.36 | -74.55; -34.17 | -21.08 | -21.91; -20.25 |
| Pancreas weight (g) | * | | * | | 1.81 | 1.68; 1.94 |
| Criterium successful isolation (IEQ) | * | | * | | -0.01 | -0.01; -0.01 |
| Preservation fluid (TLM vs UW) | 788.36 | 564.52; 1012.20 | 237.05 | 56.12; 417.98 | 32.88 | 31.33; 34.43 |
| **Isolation** | | | | | | |
| Brand of collagenase (Serva vs other) | * | | 1290.56 | 1112.85; 1468.26 | * | |
| Brand of collagenase (Sigma vs other) | * | | -650.88 | -943.12; -358.65 | * | |
| Purification (continuous vs discontinuous) | * | | * | | * | |
| Purification (Ficoll vs other) | * | | NS | | * | |

* Data available in less than 50% of the studies or not significant in univariate analysis

studies would be standardized we could possibly identify other factors and make more accurate estimation of the independent effect of these factors. To illustrate the necessity of these standardized reports, we have looked at the missing variables in our analysis. In univariate analysis 66.3-87.5% of the available pancreata were analyzed on the effect on pre- and post-purfication yield or percentage of successful isolation. In multivariate analysis this percentage was only 10.3-20.6%, due to missing data on at least 1 of the variables included. This indicates that the studies differ to such a great extent in the variables that they report, even when we selected only those variables that were reported in most studies.

Standardized reporting of the factors in all studies in the future on a minimal set of variables would also lead to a better fit of the model used in any meta-analysis. In the current analysis on post-purification yield 19% of the variance in islet isolation outcome could be explained by the included variables. In pre-purification islet yield and proportion of successful isolations, this percentage was better, but still only 50% of the variance could be explained. This suggests that besides the reported variables other factors also influence isolation outcome.

In conclusion, this study identified donor, pancreas and isolation relating factors that influence islet isolation yield. However, standardized reports of these factors are lacking, and are needed to get more reliable evidence. To improve the power and provide better comparisons in future research, standardized reporting of these factors are recommended.

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