



# Ischemic Preconditioning Improves Islet Recovery After Pancreas Cold Preservation

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## ABSTRACT

Increasing evidence supports the beneficial effects of ischemic preconditioning (IPC) of organs on subsequent ischemia. The aim of this study was to assess the effects of IPC of the pancreas on islet cell recovery after cold preservation using a rat model. The pancreas was deprived of perfusion (celiac artery and superior mesenteric artery occlusion) for 10 minutes followed by 10 minutes of reperfusion. Islet isolation was performed after 18 hours of cold ischemia. Glands undergoing IPC yielded significantly greater numbers of islets than controls. Following overnight culture, a significantly greater proportion of islets was recovered from IPC-treated pancreata. Microarray genomic analysis of pancreatic tissue revealed a significant differential expression of ~600 unique mRNA strands within IPC pancreata compared to only <100 unique mRNA strands within non-IPC pancreata (>2-fold change;  $P < .05$ ). Proteomic analysis revealed significant differential expression of at least 5 proteins (>1.5-fold change;  $P < .05$ ) within the IPC vs control group. Our data indicated that IPC of the pancreas prior to cold preservation was associated with improved islet cell recovery after cold ischemia. IPC of the pancreas may represent a viable therapeutic intervention to increase islet transplantation success from a single donor and to maximize organ utilization.

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**C**URRENT HURDLES to whole pancreas and islet transplantation include the limited number of organs suitable for transplantation.<sup>1</sup> Pancreas ischemia and cold preservation are associated with organ injury that may negatively impact the quality of the organ as well as the number and quality of islets obtained after isolation.<sup>2</sup> Increasing evidence supports the beneficial effects of ischemic preconditioning (IPC) of organs on subsequent ischemia.<sup>3–6</sup> IPC may result in the activation of cytoprotective “survival” pathways resulting in improved organ preservation. The aim of this study was to assess the effects of IPC of the pancreas on islet cell recovery after cold preservation.

## MATERIALS AND METHODS

Animals purchased from Harlan Laboratories (United States) were used in compliance with the IACUC. Male Lewis rats were used as pancreas donors. To test the effects of IPC, the pancreas was deprived of perfusion by applying a microvascular clamp on the celiac and the superior mesenteric arteries for 10 minutes followed by a reperfusion period of 10 minutes prior to cold preservation. Organs were perfused with and stored in University of Wisconsin (UW) solution. Following 18 hours of cold preservation, islet

isolation was performed using Liberase (Roche) digestion followed by Euro-Ficoll density purification (Mediatech-Cellgro), as previously reported.<sup>7</sup> Islet yields and recovery after overnight culture were measured from control and IPC-treated pancreata.<sup>8</sup> Islet function was assessed in vivo by transplanting isolated islets into immunodeficient (athymic nu/nu) mice rendered diabetic with streptozotocin (200 mg/kg; Sigma-Aldrich).<sup>9</sup> Proteomic analysis was performed using 2-DIGE and MALDI-TOF (Applied Biomics; Hayward, Calif, United States). Microarray genomic analysis was performed on whole rat genome using Applied Biosystems.

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## RESULTS

Glands undergoing IPC yielded significantly greater numbers of islets than controls (1.3-fold;  $P < .05$ ; Table 1). Islet cell loss after overnight culture was used as a surrogate indicator of islet quality: a significantly greater proportion of islets was recovered from IPC-treated compared with control pancreata (1.4-fold;  $P < .01$ ; Table 1). In vivo functional status, assessed by reversal of diabetes in mice after islet transplantation, demonstrated comparable function between IPC and control groups (not shown).

Preliminary microarray genomic analysis of pancreatic tissue revealed a significant differential expression of 602 unique mRNA strands within IPC pancreata compared to only 95 unique mRNA strands within non-IPC pancreata ( $>2$ -fold change;  $P < .05$ ). Preliminary proteomic analysis revealed significant differential expression of at least 5 proteins ( $>1.5$ -fold change;  $P < .05$ ) within the IPC versus control group.

## DISCUSSION

One limitation of clinical islet transplantation is the inability to obtain a sufficient number of islets from one donor pancreas to reverse diabetes in a recipient.<sup>1</sup> Our data indicated that IPC of the pancreas prior to cold preservation was associated

with improved islet cell recovery after cold ischemia. The use of IPC of the pancreas may represent a viable therapeutic intervention to increase the success of islet transplantation from a single donor. A better understanding of the underlying mechanisms of IPC-mediated cytoprotection in this model may assist in developing targeted interventions to maximize organ utilization for transplantation.

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**Table 1. Effects of IPC of the Pancreas on Islet Yield and Recovery After Culture**

	Control (n)	IPC (n)	P
Islet yield (per donor pancreas)	597 ± 104 (31)	774 ± 104 (28)	<.05
Islet recovery after culture	35 ± 10 (6)	52 ± 9 (6)	<.01