Use Of Animal Models To Assess Islet Graft Function

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Experimental Transplantation

- **Samples of the isolated human islets can be implanted into normoglycemic and/or hyperglycemic animals (nude mice or rats)**
  - **- freshly isolated**
  - **- after culture**

Experimental Transplantation

- **Allows for evaluation, at different times after implantation**
  - **- short term (<3-4 days)**
  - **- long term**

Animals VS Humans

- Relevance of diabetes models
- Absence of autoimmunity
- Number of islets needed for cure
- Implantation organs
- Glucotoxicity
- Lipotoxicity

Implantation Site

- **Renal capsule**
- **Liver (intraportally)**
- **Spleen**
- **Omental pouch**

- **Other**

Experimental Transplantation

SHORT TERM

- **Ability to induce normoglycemia**
- **β-cells**
- **Other cells**
- **Hormone release**

LONG TERM

- **Cellular composition of graft**
- **Revascularization**
- **Reinnervation**
- **Amyloid deposits**

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Ability To Induce Normoglycemia

- **Number of islets needed?**
- **When after implantation does normo-glycemia occur?**
- **Dependent on implantation organ?**

Experimental Transplantation

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Cellular Composition

- **Immunostaining of histological sections**
- **Evaluation of number of different cell types and degree of fibrosis**

- **Fibroblasts/myofibroblasts (stellate cells)**
- **Macrophages/Dendritic cells**
- **Nerve cells/Schwanns cells**
- **Endothelial cells/pericytes**

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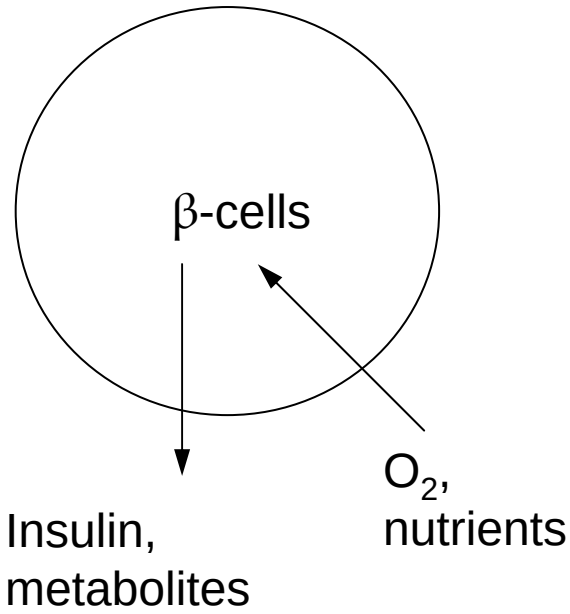
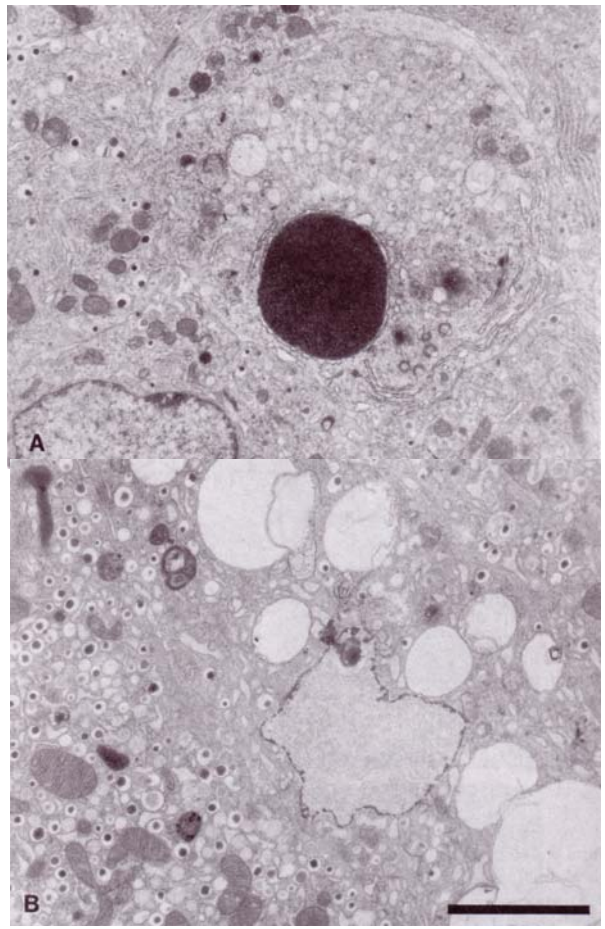
Endothelial Cells

- **Vascular density, number of endothelial cells**
- **(Blood perfusion)**
- **pO_2**
- **Metabolism**
- **Capillary blood pressure**
- **Lymphatics**

- **Vascular dysfunction**

Day 0-1 Post-Tx





Apoptosis

Necrosis

Davalli et al, Diabetes 45:1161, 1996

Revascularization

- **Revascularization initiated within 1-3 days and concluded within 7-14 days.** ***Menger et al Diabetes 38(Suppl 1):199, 1989***

- **VASCULOGENESIS**

Formation from angioblasts

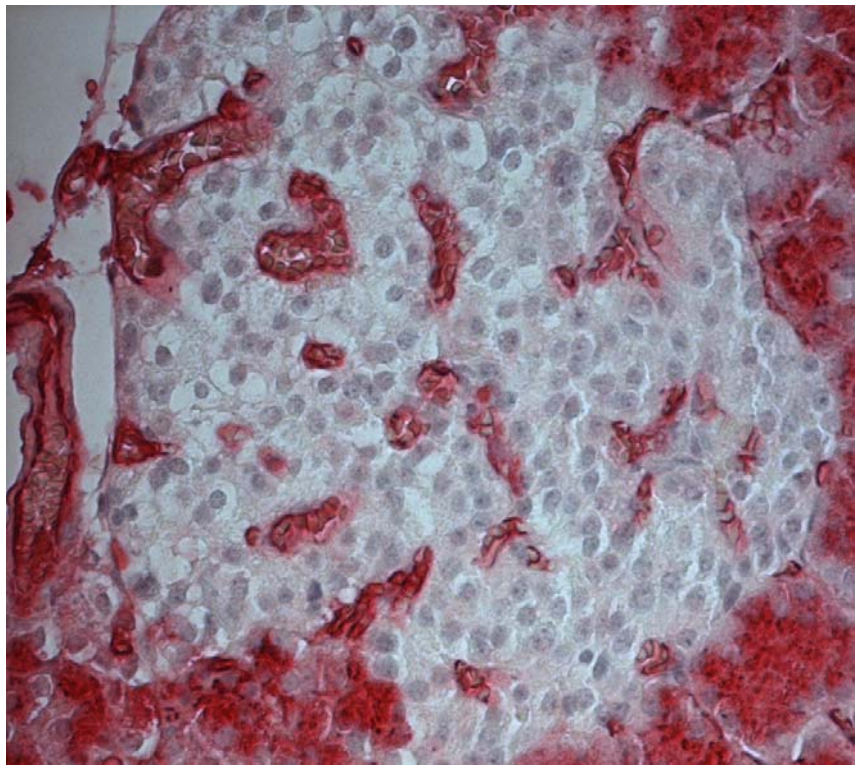
- **ANGIOGENESIS**

- **Sprouting**
- **Intussusceptive growth**

Vascular Density

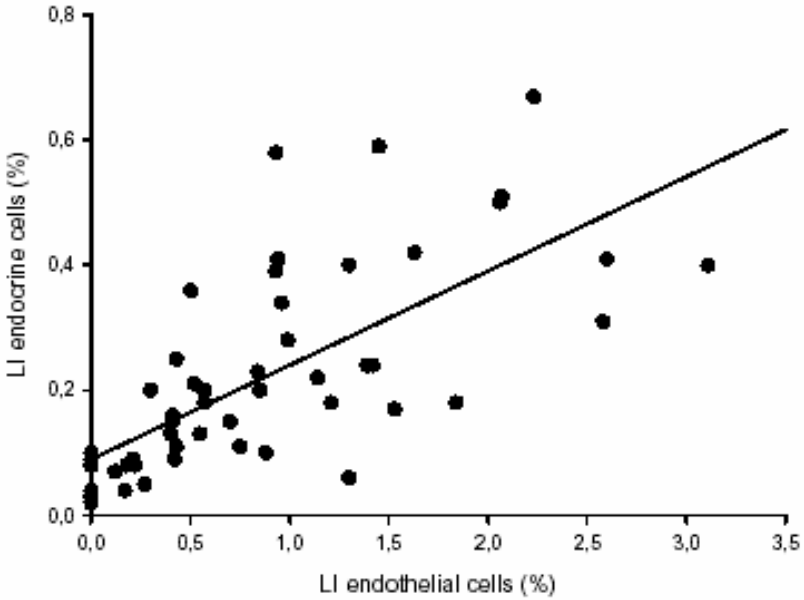
- **Bandeiraea simplicifolia (BS-1)**
- *Carlsson et al, JCEM 87:5418, 2002*

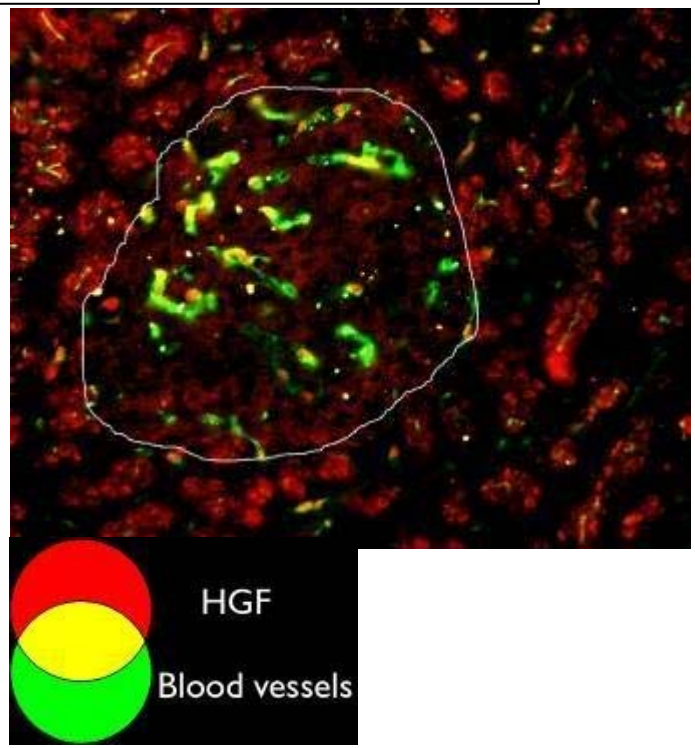
- **von Willebrand actor**
- **Ulex lectin**
- **CD31**



Decreased Growth Potential?

Pregnant rats, gestational day 15

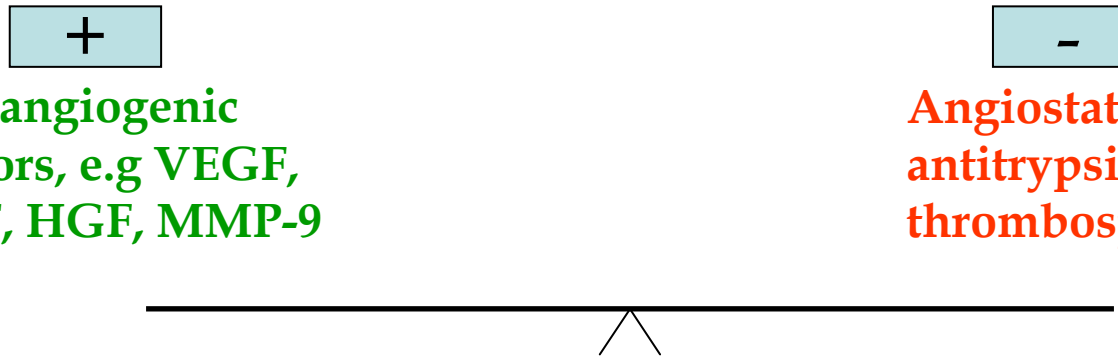




Johansson M et al, FASEB J, 19: A1713, 2005

Improve Islet Revascularization

- **Stimulation of Islet Angiogenesis**



Vasir et al, Diabetologia 43:763, 2000

Vasir et al, Transplantation 71:924, 2001

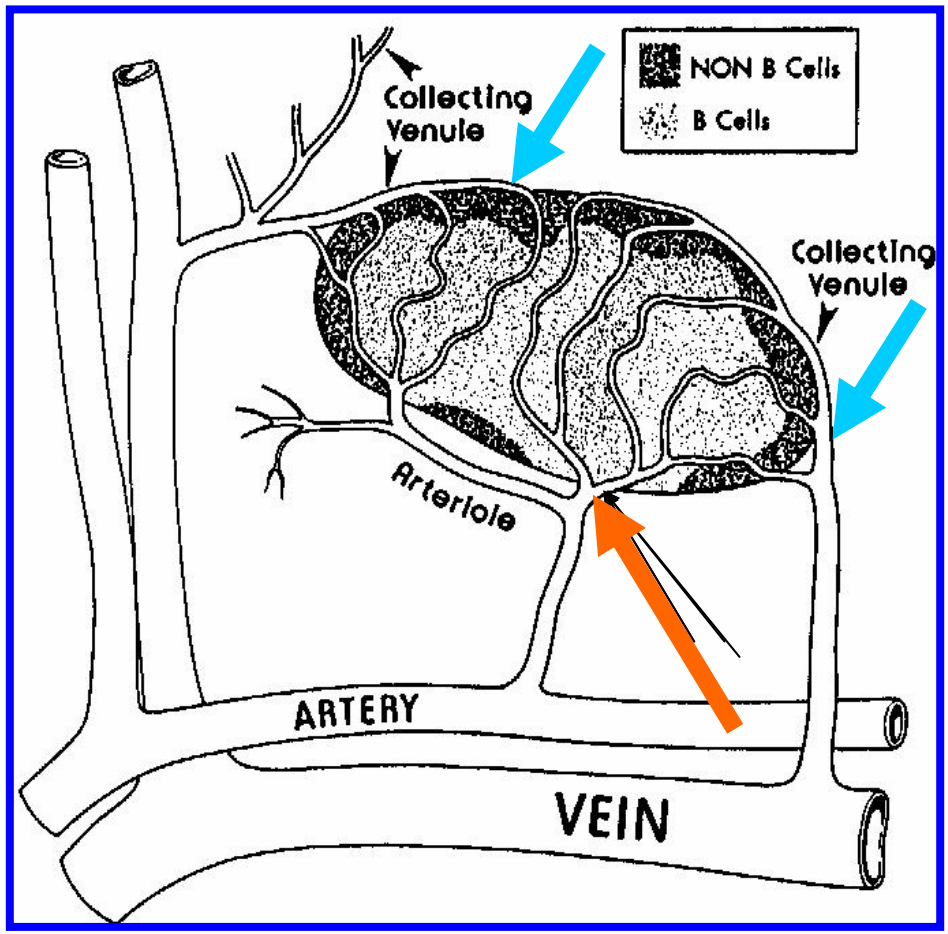
Bergers et al, Nat Cell Biol 2:737, 2000

Lou et al, Diabetes 48:1773, 1999

Tillmar and Welsh JOP 5:81, 2004

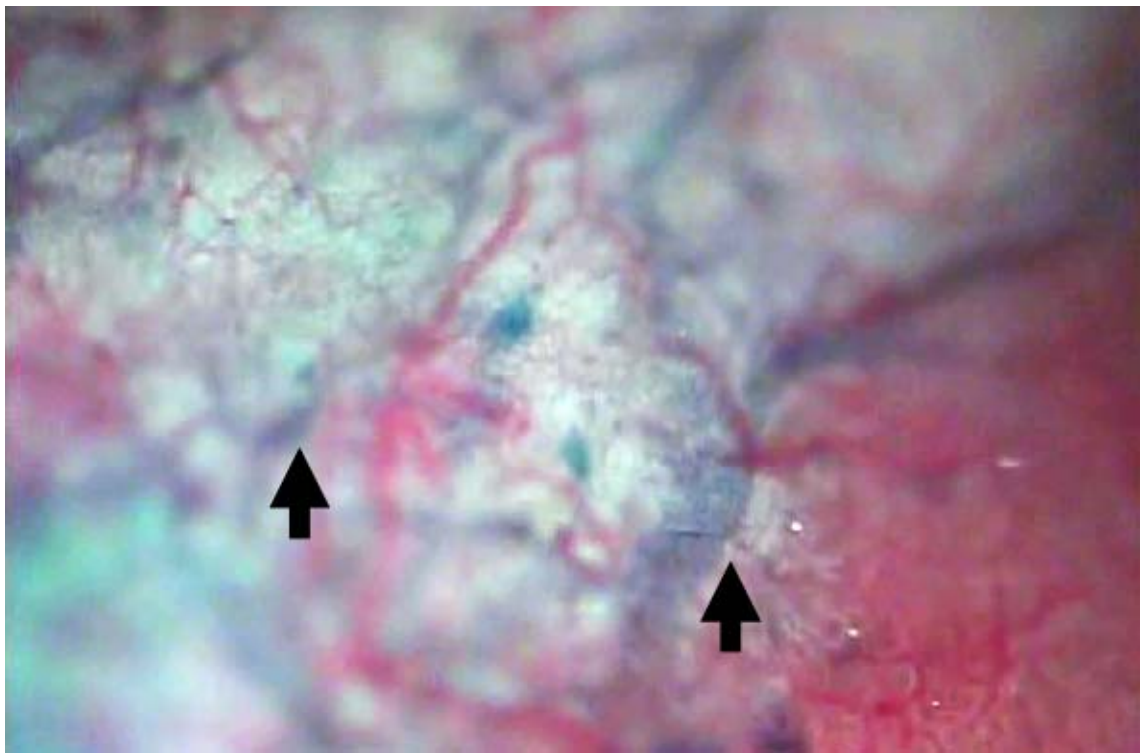
Mattsson et al, Pancreatology, in press

Vascular Organization



Bonner-Weir and Orci, Diabetes 31:883, 1982

In Vivo Microscopy



In vivo microscopy with Evans Blue

Endothelial Cells

- **Vascular density, number of endothelial cells**
- **(Blood perfusion)**
- **pO_2**
- **Metabolism**
- **Capillary blood pressure**
- **Lymphatics**

Blood Perfusion

- **In vivo microscopy with cross correlation**
- **Laser-Doppler flowmetry**
- **Hydrogen gas clearance**

- **All these techniques can be used on the same graft**

Endothelial Cells

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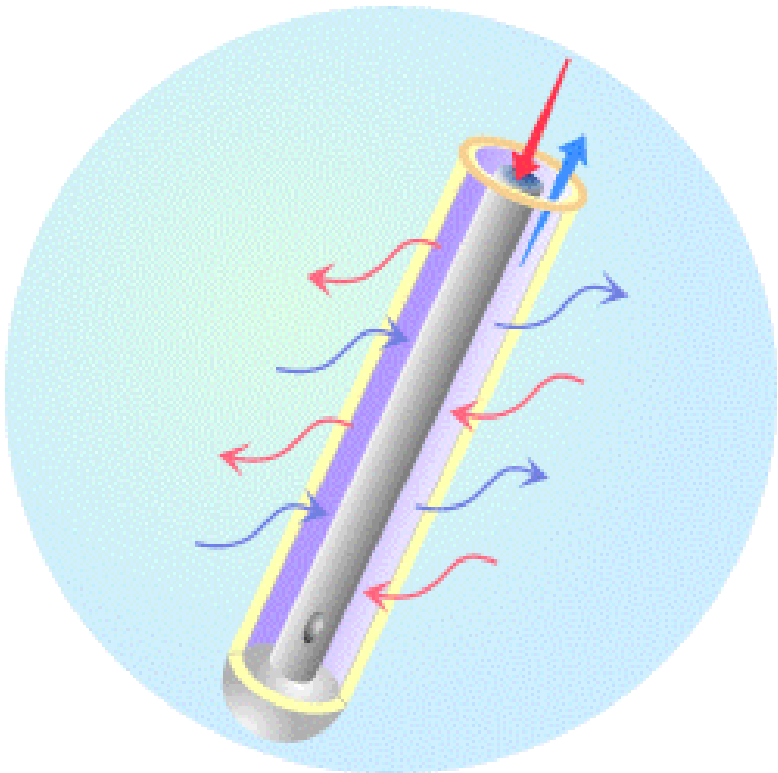
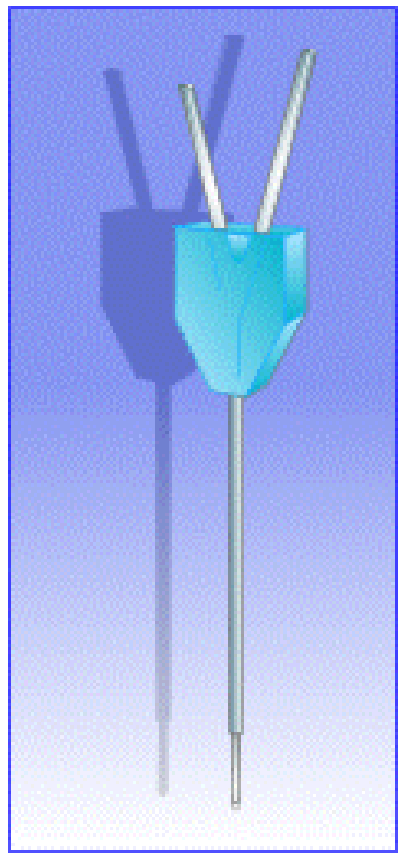
Microdialysis

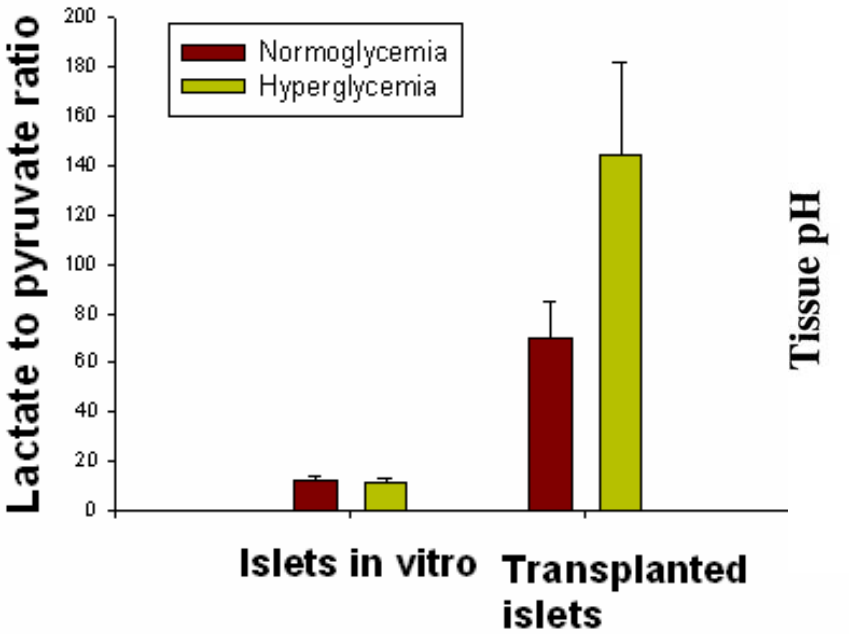




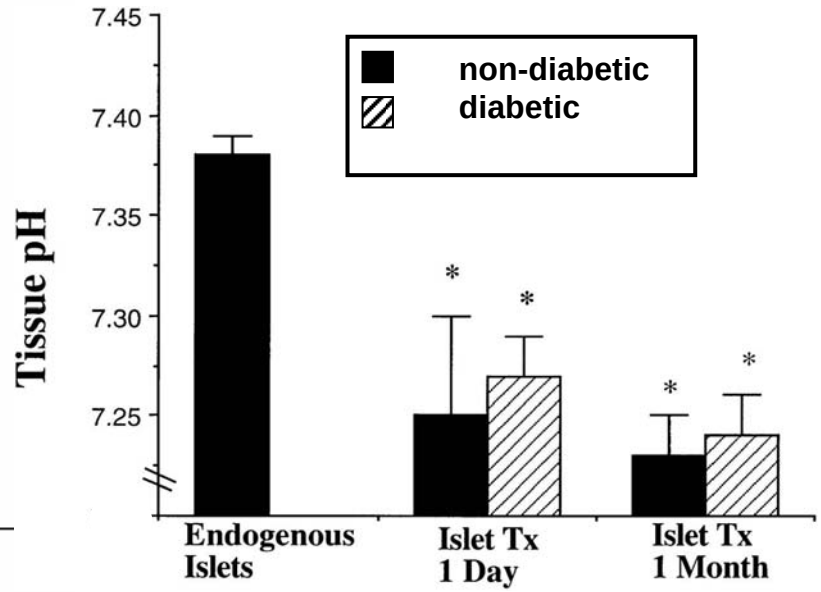
Diameter 200 µm

Anaerobic Metabolism

One-month-old islet graft



Carlsson et al, Surgery 132:487, 2002



Carlsson et al, Am J Physiol 284:E499, 2003

Endothelial Cells

- **Vascular density, number of endothelial cells**
- **(Blood perfusion)**
- **pO_2**
- **Metabolism**

- **All these parameters can be studied in the same graft**

Experimental Transplantation

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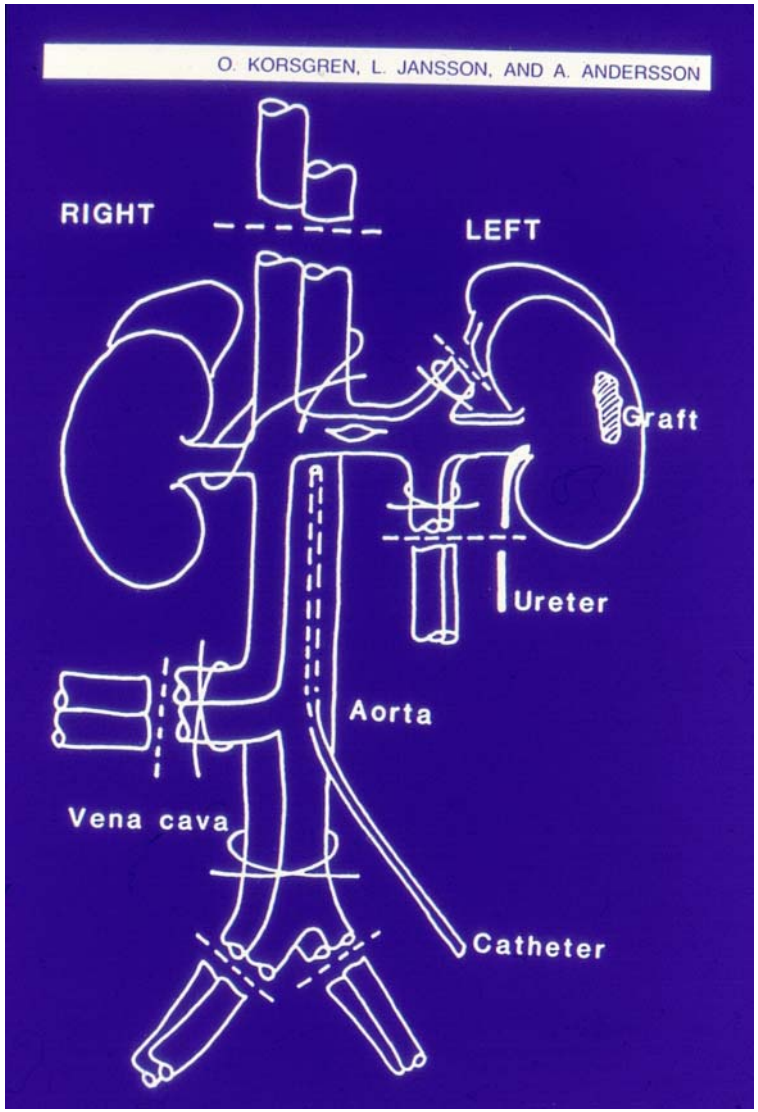
Studies Of Hormone Release

- **Ability to induce normoglycemia**
- **Removal of graft and in vitro perifusion (excludes livers)**
- **Perfusion of graft-bearing organ (graft vasculature)**

- **The latter two allows for studies also of glucagon and somatostatin**

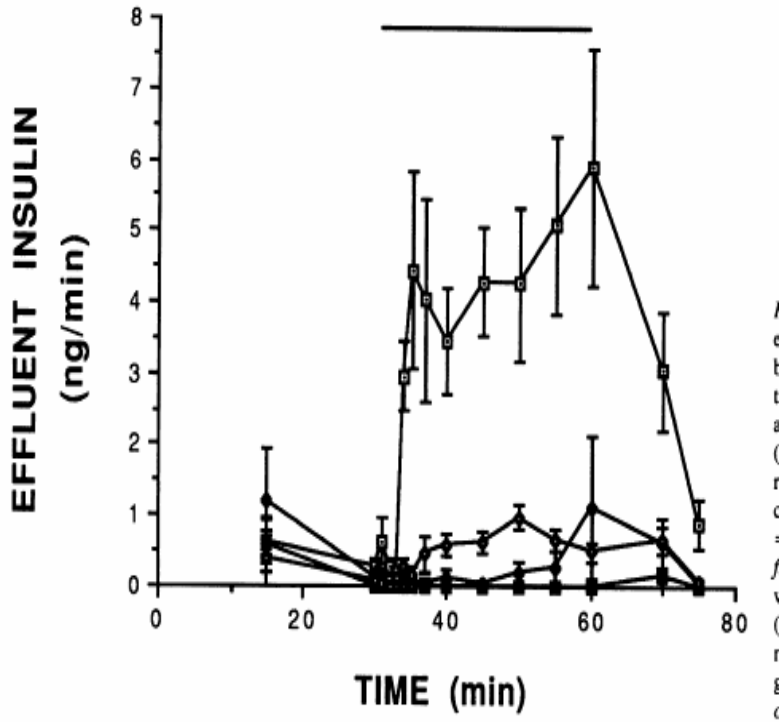
Kidney Perfusion



Insulin Release Human Islets

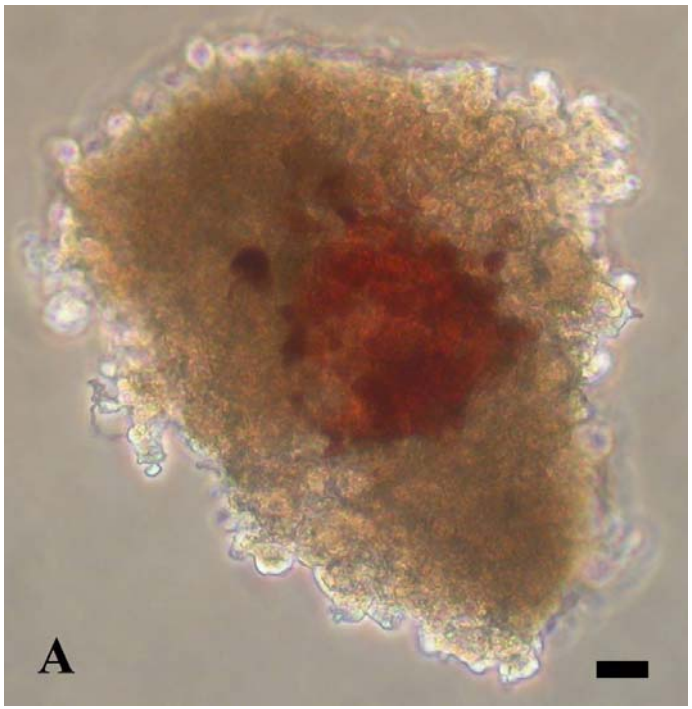
- **Biphasic release from islets in normoglycemic recipients**
- **Impaired release in hyperglycemic recipients**

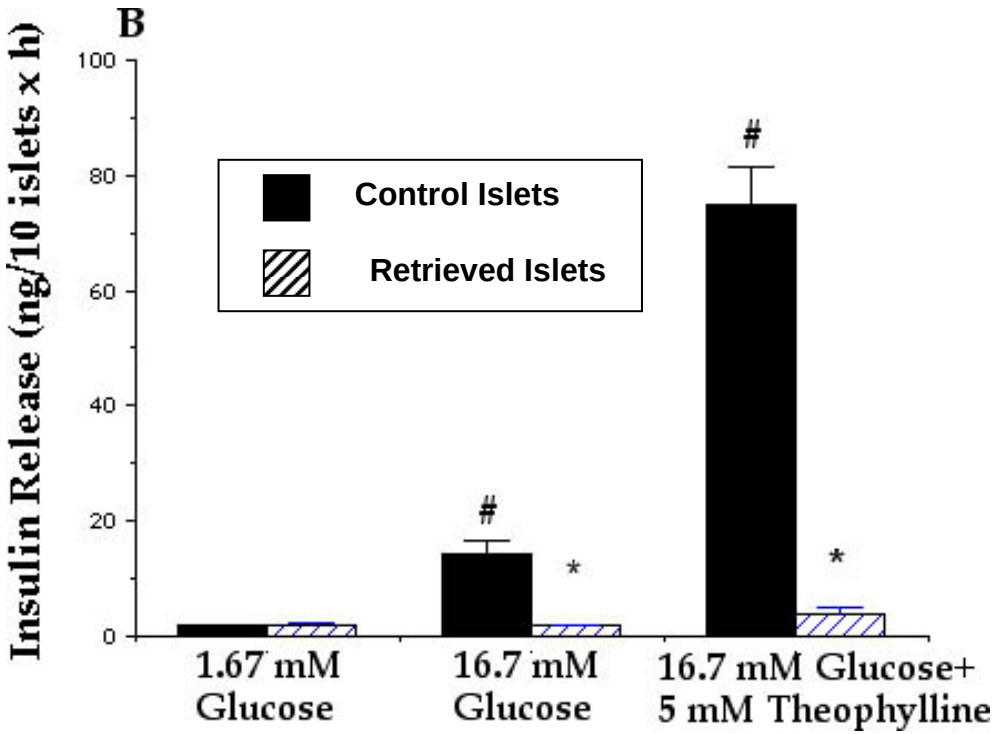
- *Jansson et al; J Clin Invest 96:721, 1995*



Retrieved Islets from Liver

One-month-old islet grafts



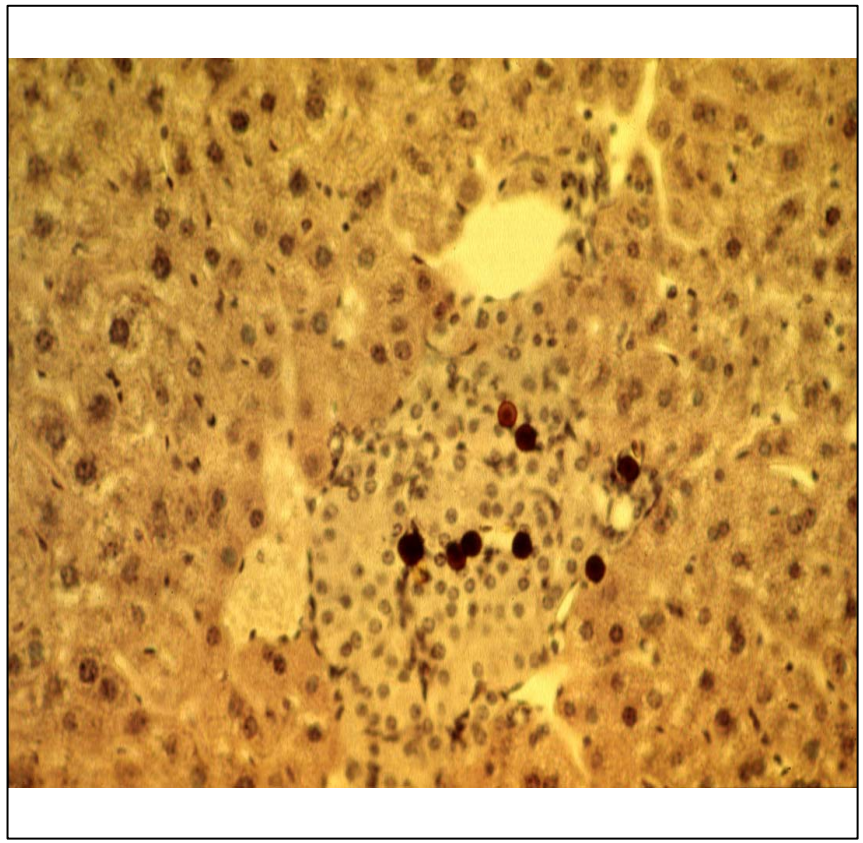


Retrieved islet visualized by Neutral Red

Mattsson et al, Diabetes 53: 948, 2004

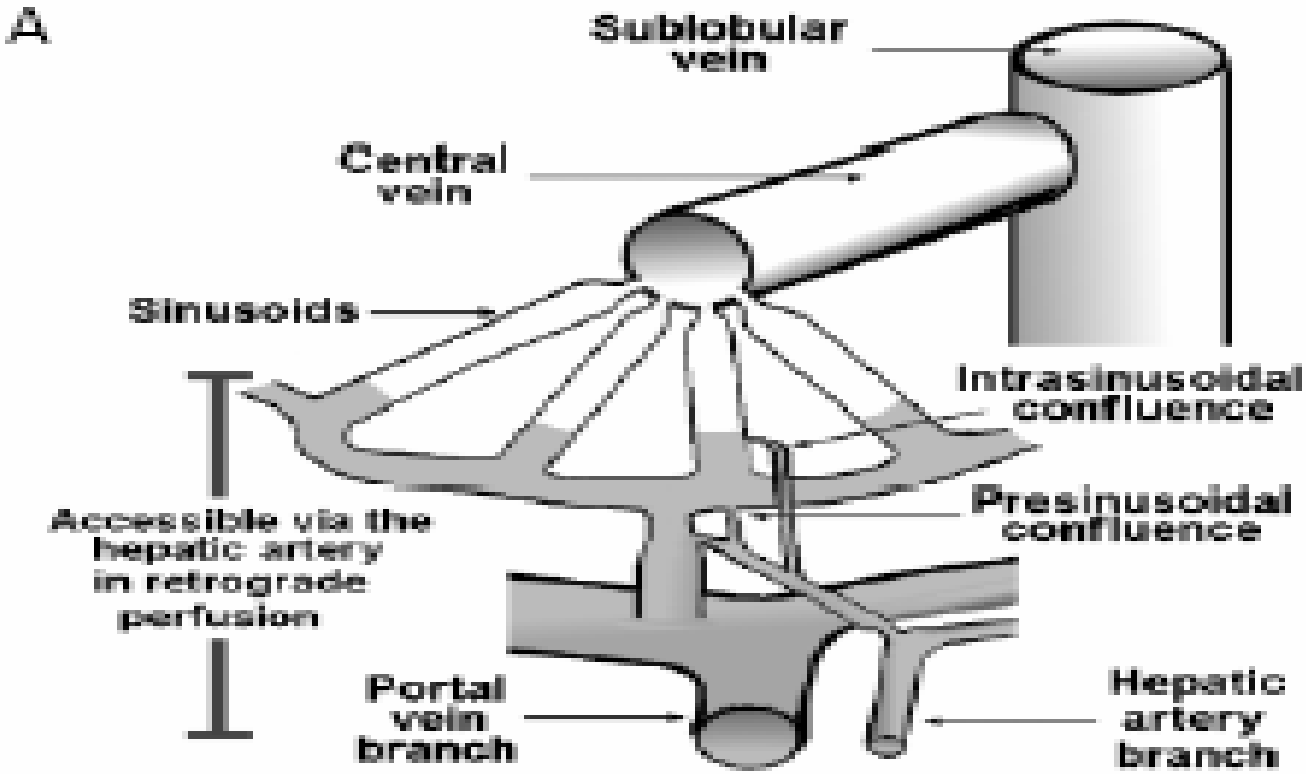
Intrahepatic Graft

- **Revascularization occurs from hepatic artery, and only to a minor extent from portal vein**



Andersson et al, Diabetes 38(Suppl 1): 192, 1989

Liver Vascular Anatomy



Botini et al, Liver Int 25:861, 2005

Intrahepatic Grafts

- **Morphological studies to asses the degree of fragmentation**
- **New in vivo imaging techiques;**

  -MRI

  -Optiplex

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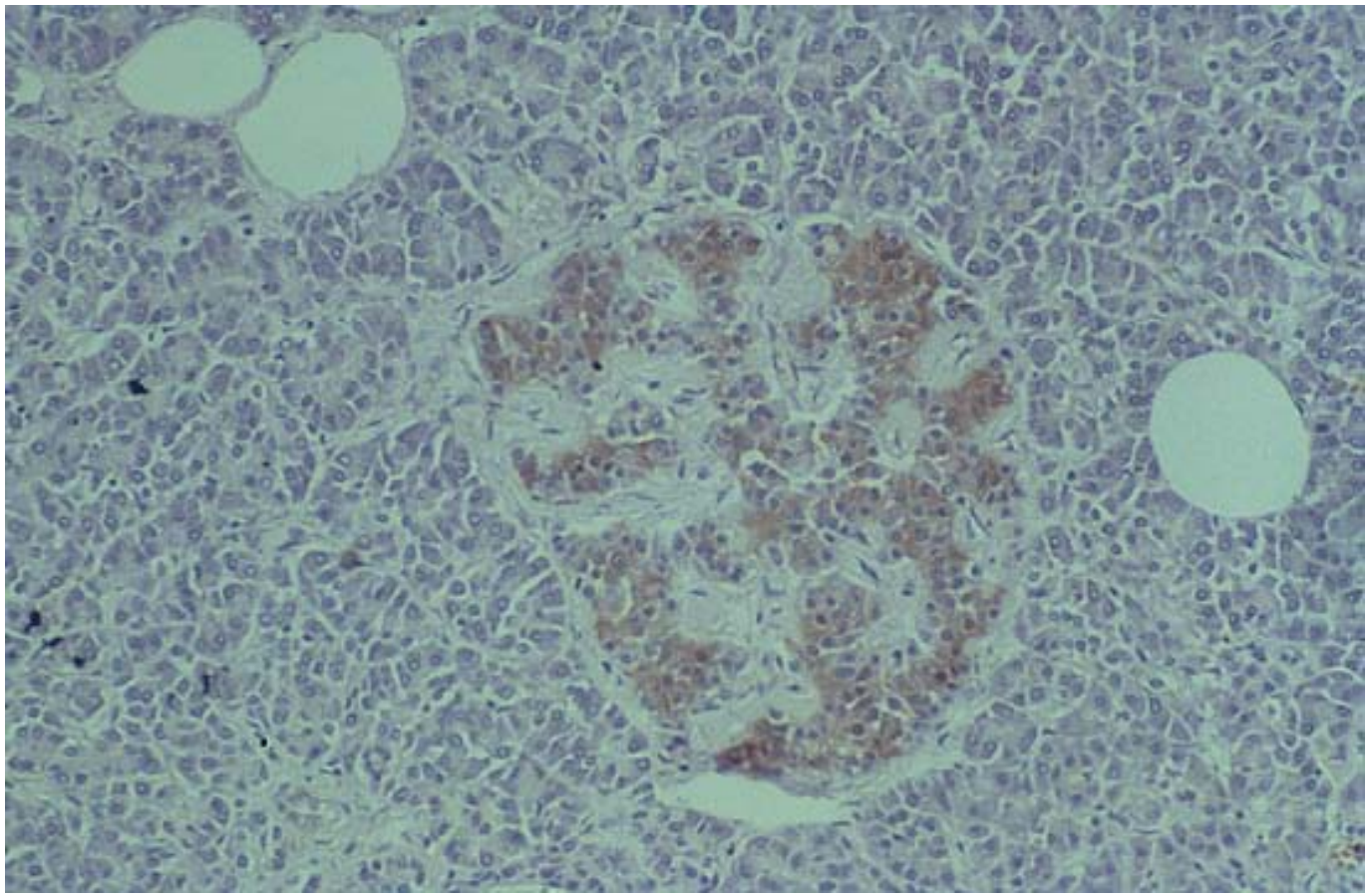
Islet Amyloid



Westermark et al; Metabolism 48:448, 1999

Amyloid Deposits

- **Amyloid occurs in 73% of human islet grafts within 2 weeks after implantation into nude mice (*Westermark et al; Metabolism 48:448, 1999*)**

- **Initially amyloid is formed intracellularly (*Westermark et al; Upsala J med Sci 108:193, 2003*)**

Conclusion

- **Experimental islet transplantation of human islets offers unique possibilities to assess graft function in both the short and long term perspective**

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