Glucose Stimulation of Cytochrome c Reduction and Oxygen Consumption as Assessment of Human Islet Quality

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Goals of Human Islet Assessment in Transplantation

- Avoid transplantation of human islets that are unlikely to be efficacious.

- Assess modifications in islet preparation and culture aimed at improving islet quality.

Electron Transport: "Vital Sign" of Cell Health

GLUCOSE

Glycolysis
TCA Cycle

1. Metabolism

3. Apoptosis

Translocation
of cyt c to
cytosol

NADH

$2e^-$

ELECTRON TRANSPORT

Cyt c

Cyt c
Oxidase

$2e^-$

OX
PHOS

**2. ATP
Generation**

O_2 $O_2^{-\bullet}$

**O$_2$
Cons.**

4. Free Radical Production

Flow Culture/Non-Invasive Assessment System
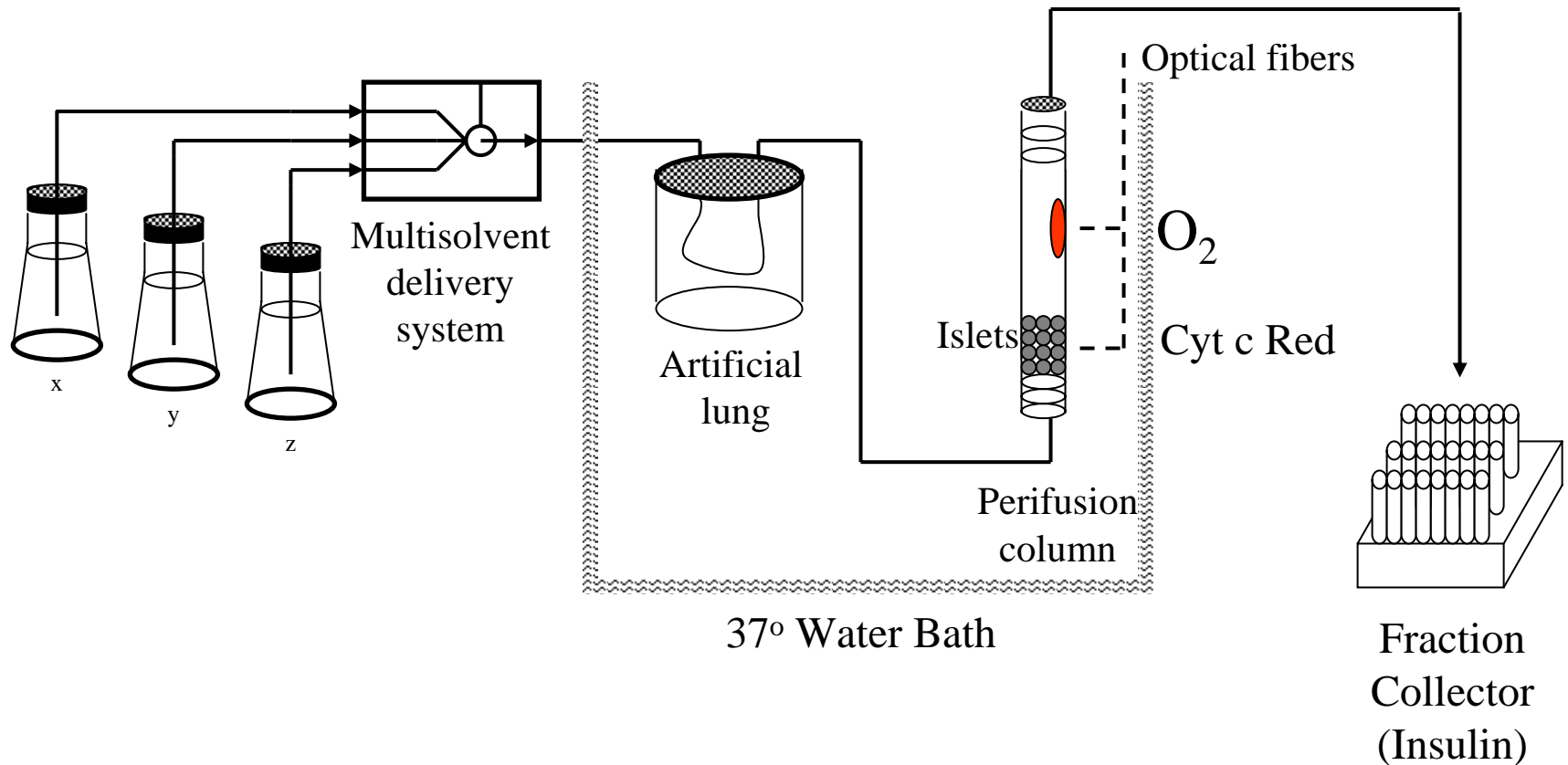
Islets in Flow Chamber: Detection of Cytochromes and Oxygen
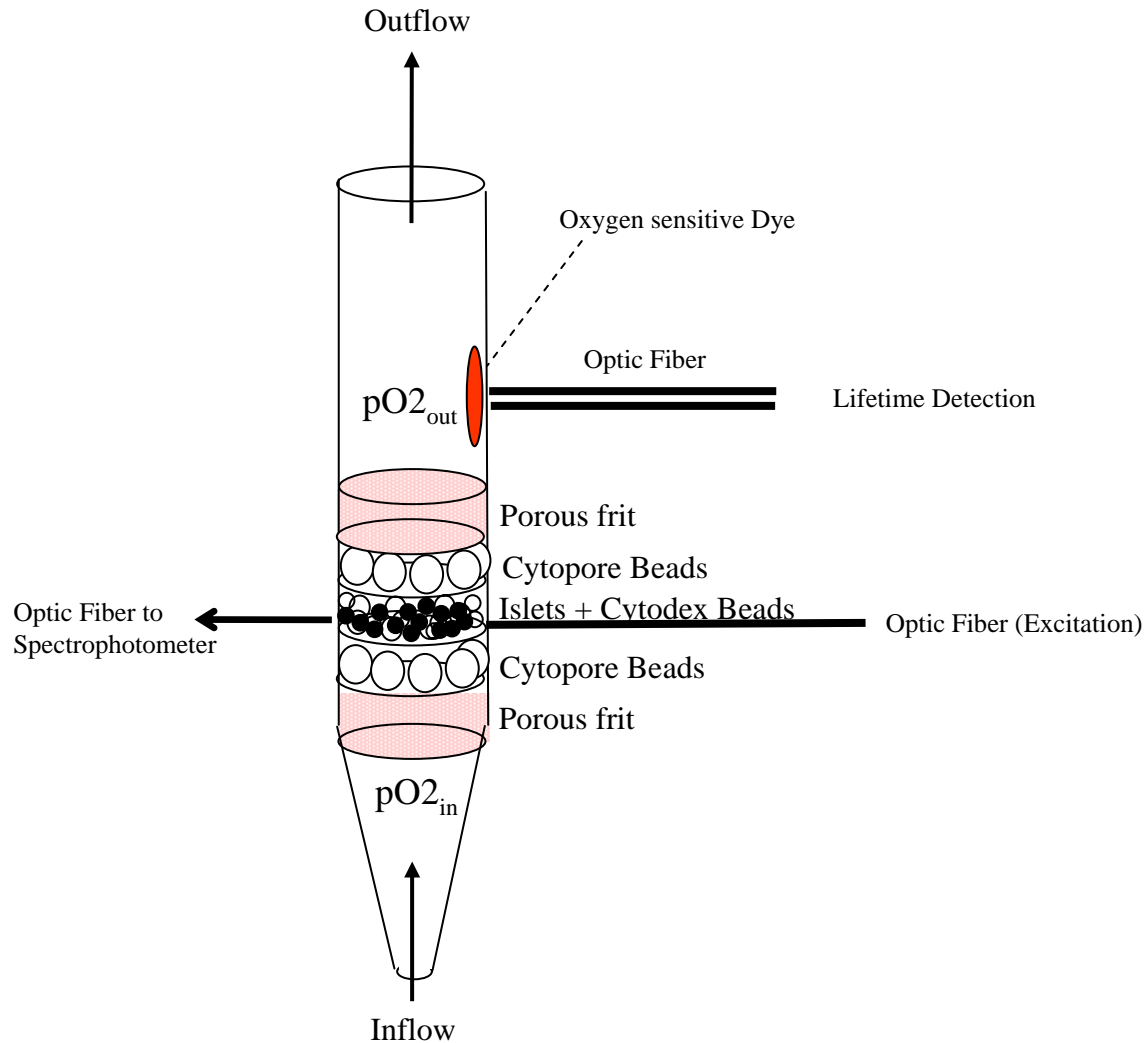
Sweet IR, DL Cook, E DeJulio, AR Wallen, G Khalil,, JB Callis, JA Reems. Regulation of ATP/ADP in pancreatic islets. *Diabetes* 53:401-409, 2004.

Glucose Responsiveness as Assessment of Human Islets

Glucose stimulation of OCR is much less variable then OCR.

Glucose will not stimulate dysfunctional islets.

Beta cells are uniquely sensitive to changes in glucose in the physiological range, so little contribution from non-beta cells.

Glucose response is a composite parameter reflecting both the beta cell number and quality.

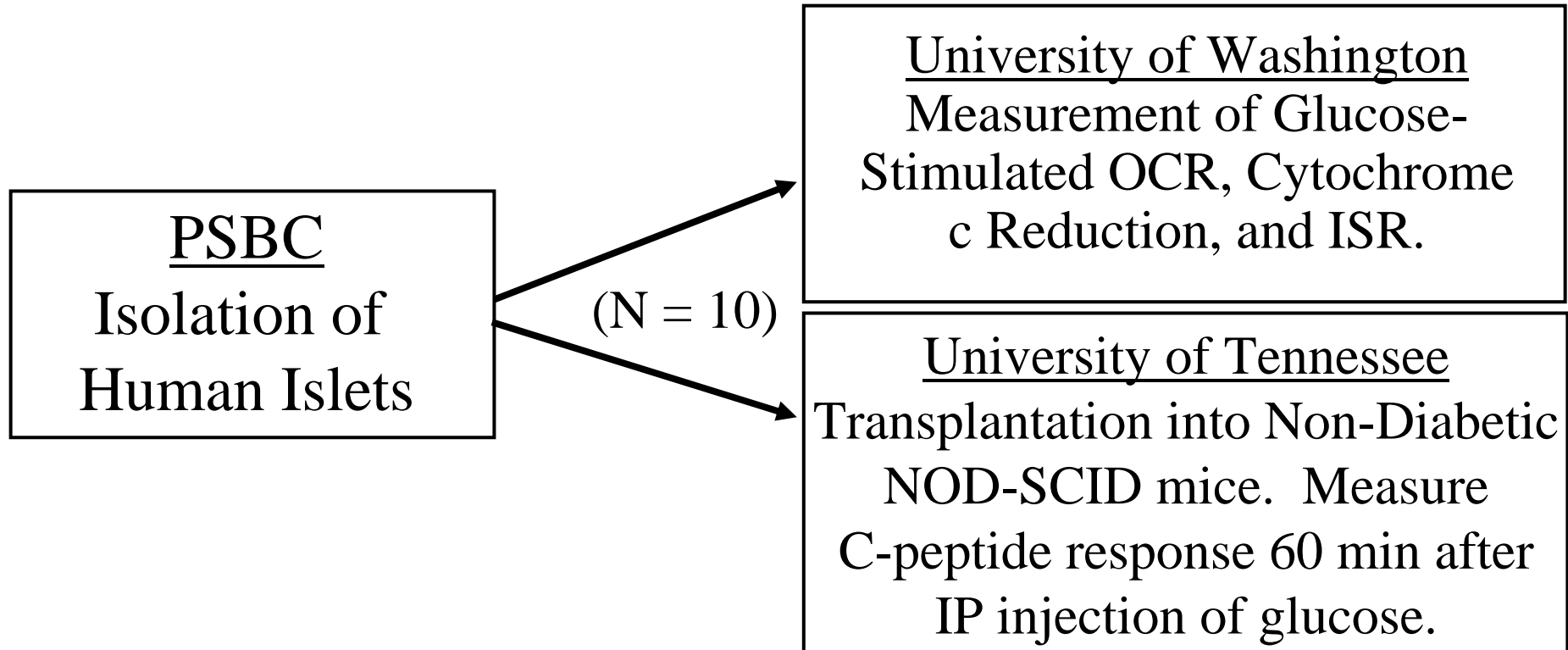# Glucose Responsiveness as Assessment of Human Islets

Hypothesis: A minimal response in cytochrome c reduction and OCR of an islet preparation due to an increase in glucose measured in vitro will predict low secretory function in vivo after transplantation.
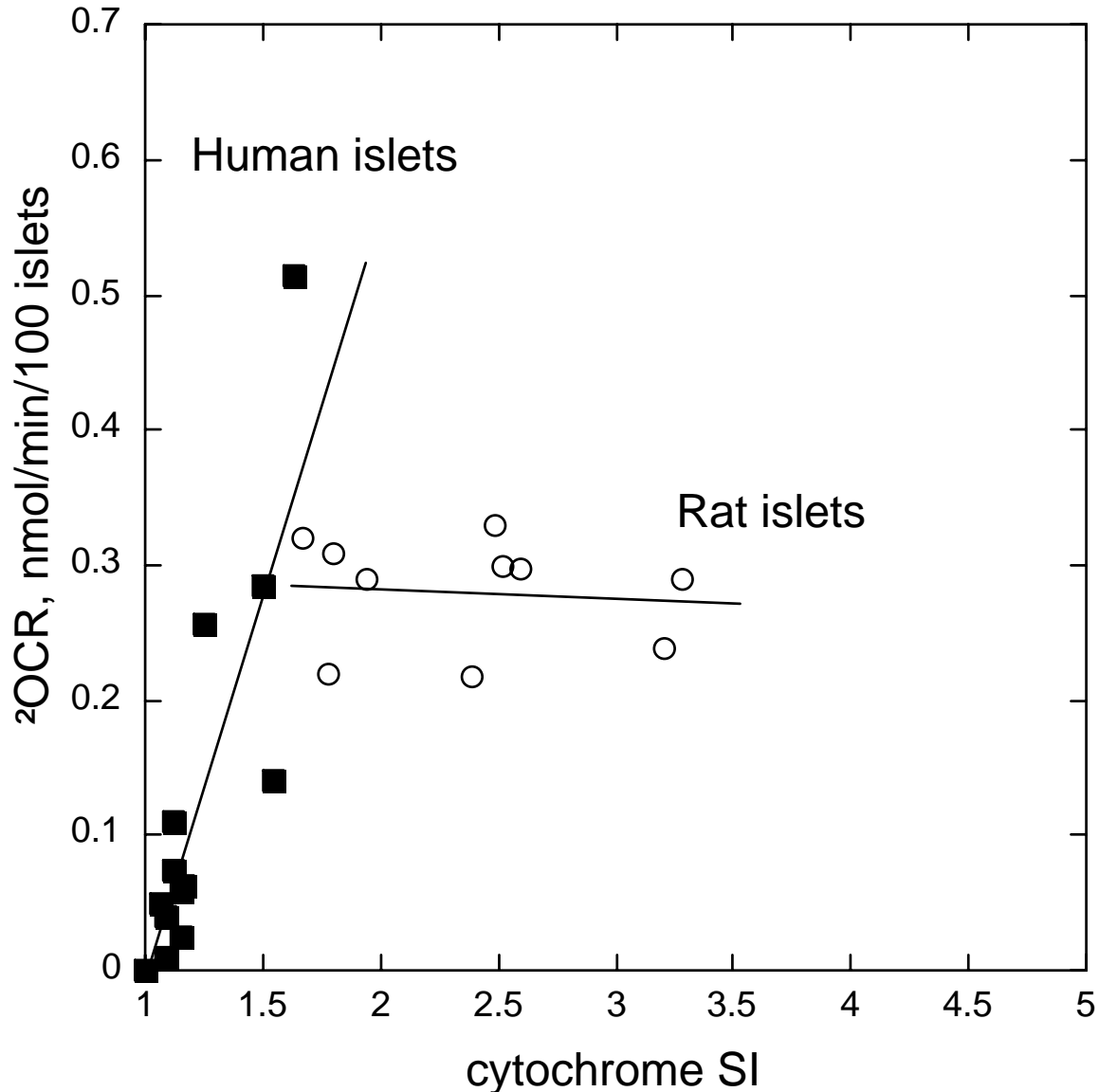
Assessment Protocol
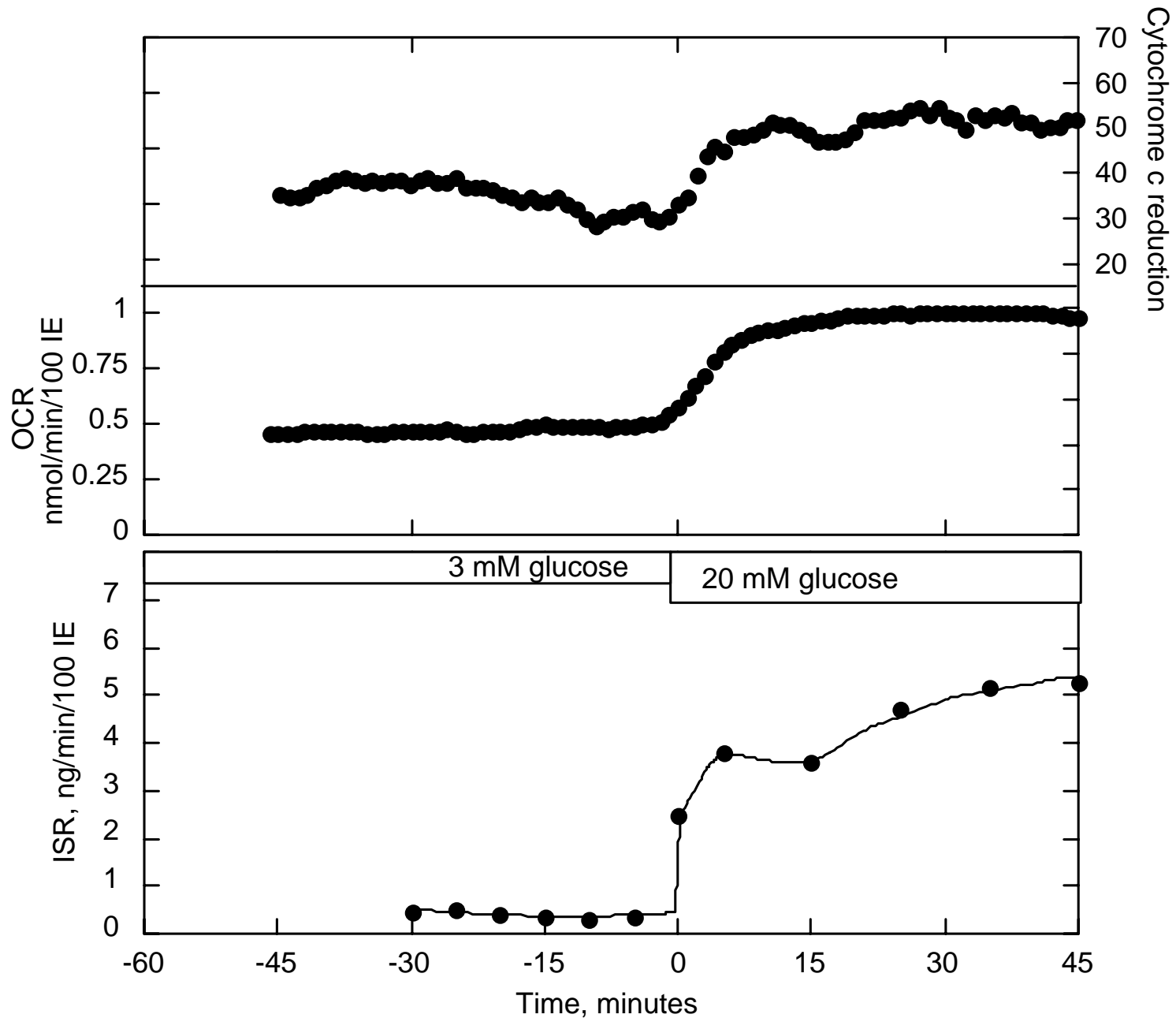
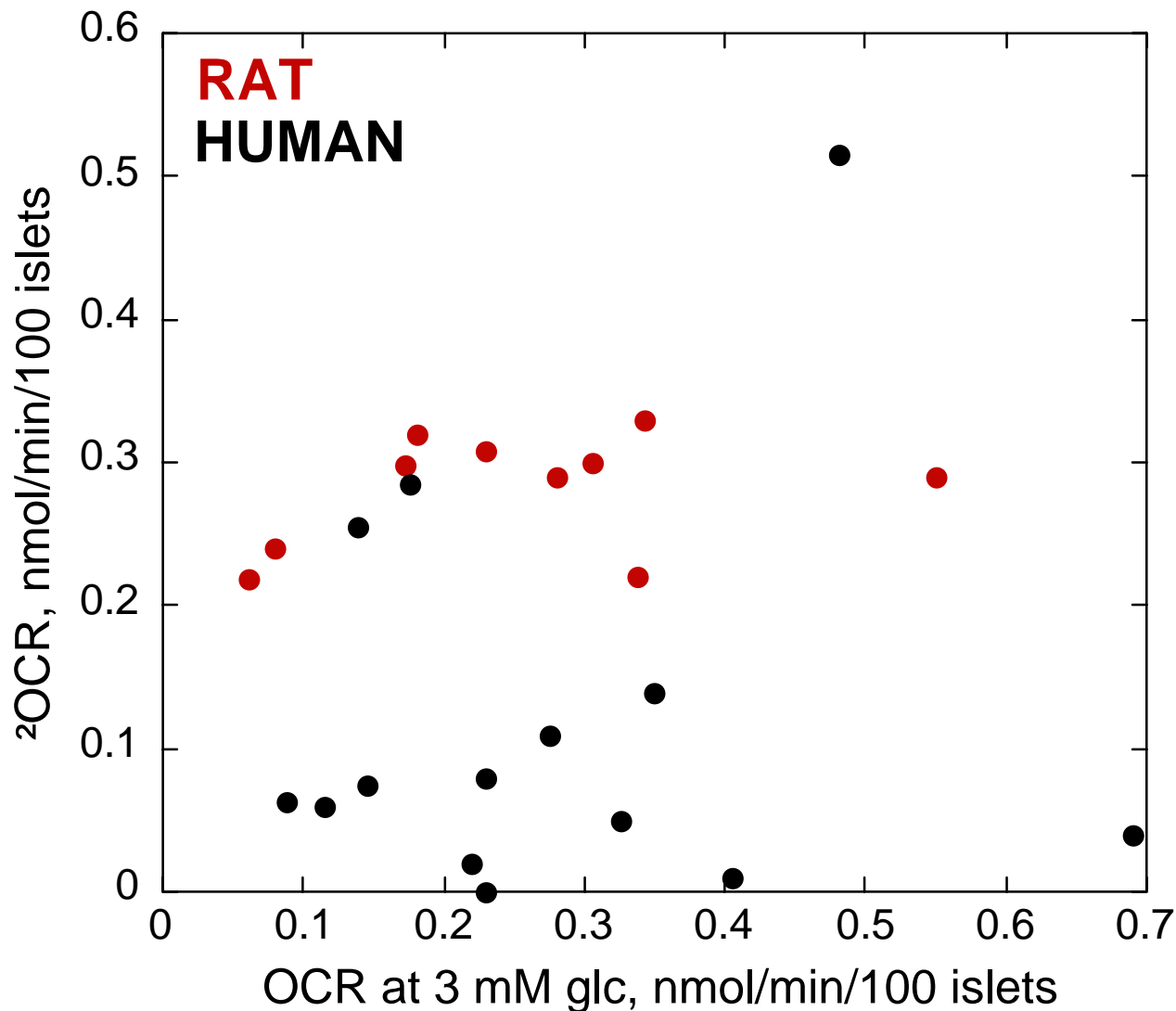# Study Design: Comparison of In Vitro and In Vivo Function of Human Islets

PSBC
Isolation of
Human Islets

(N = 10)

University of Washington
Measurement of Glucose-Stimulated OCR, Cytochrome c Reduction, and ISR.

University of Tennessee
Transplantation into Non-Diabetic NOD-SCID mice. Measure C-peptide response 60 min after IP injection of glucose.
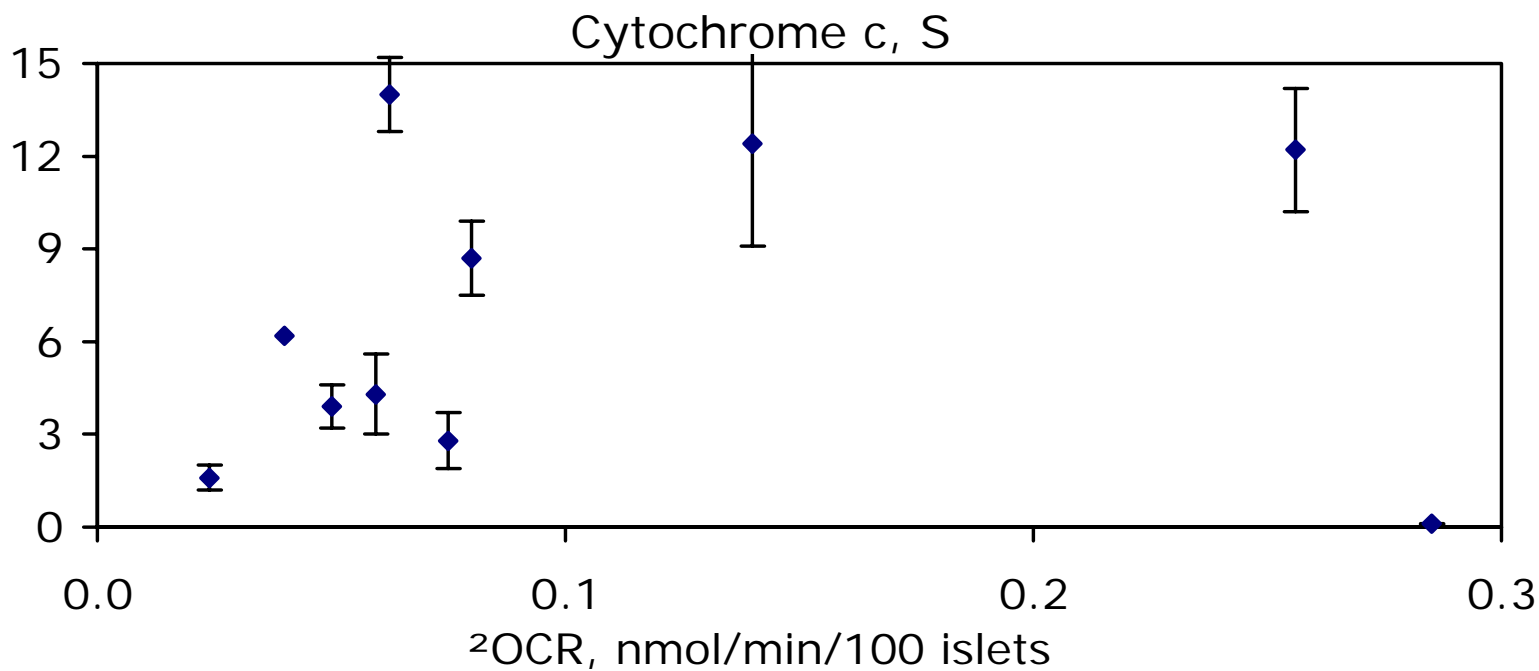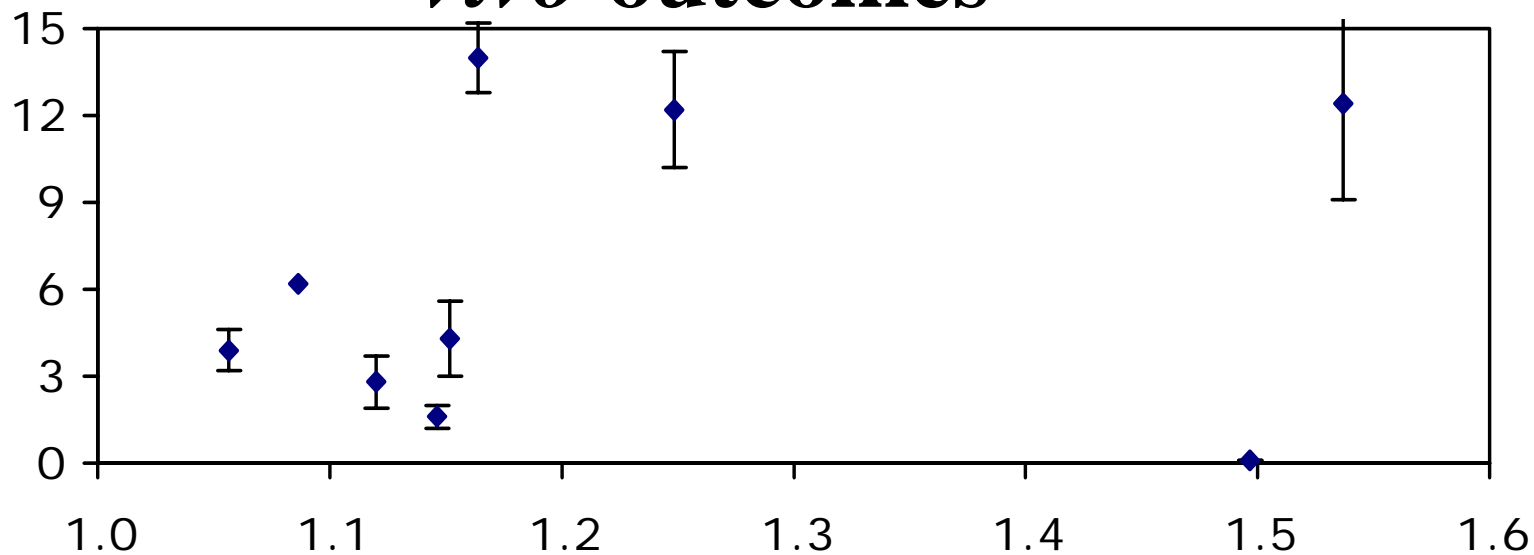
Responses in Human Islets are Less Robust and More Variable than in Rat Islets

Glucose-stimulated OCR does not correlate with OCR

Comparison of *in vitro* assessment and *in vivo* outcomes

Analysis using Receiver Operating Curves

1. Define a threshold of success with respect to the in vivo transplants.
2. Generate ROCs by plotting Sensitivity vs 1-Specificity

   **Sensitivity**: true positives/outcomes above threshold
    Specificity: true negatives/outcomes below threshold

3. Calculate the Area Under the Curve. The AUC will be 1 if the correct classification is 100%, and 0.5 if there is no predictive relationship.

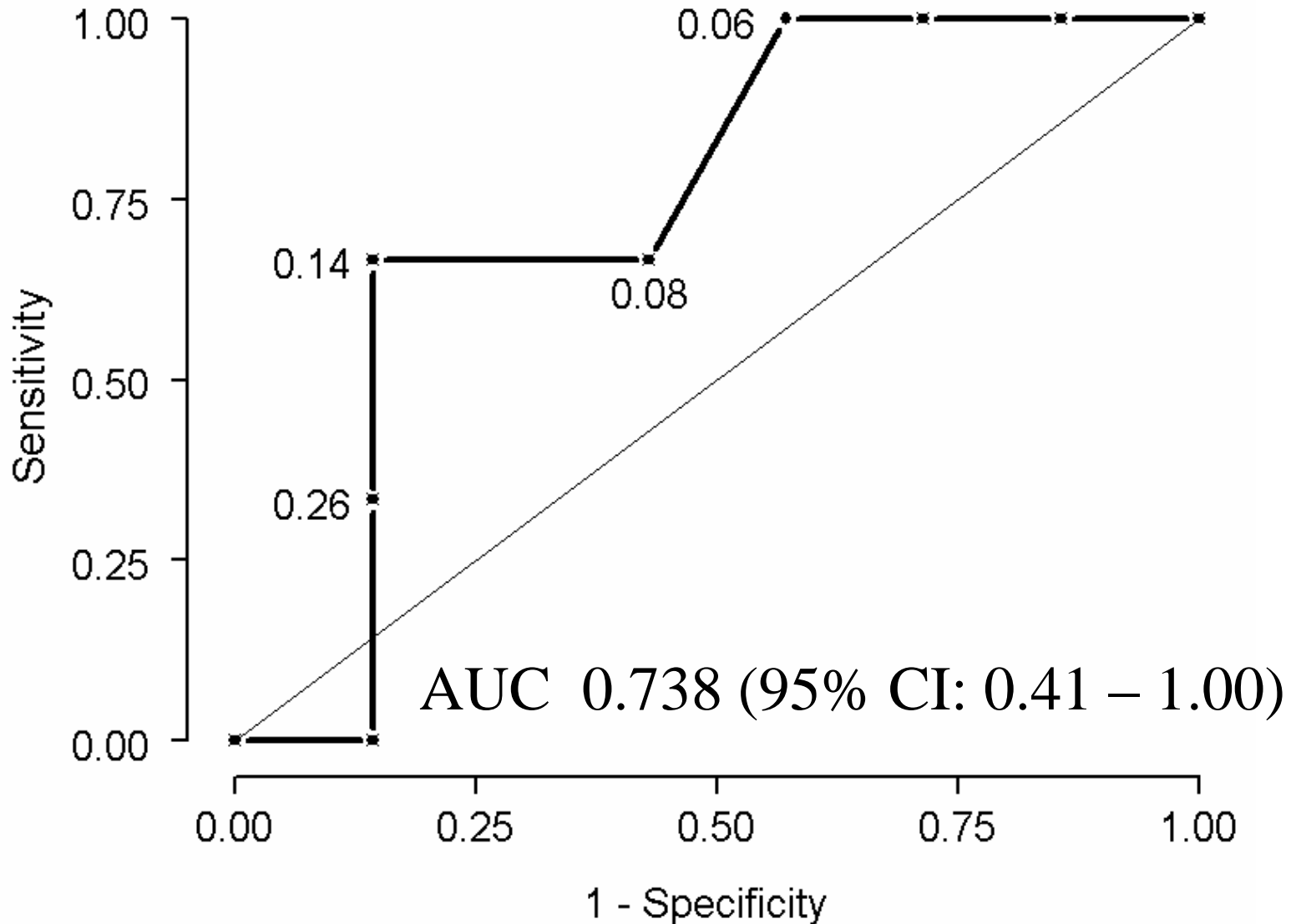# Threshold of Success With Respect To In Vivo Outcome

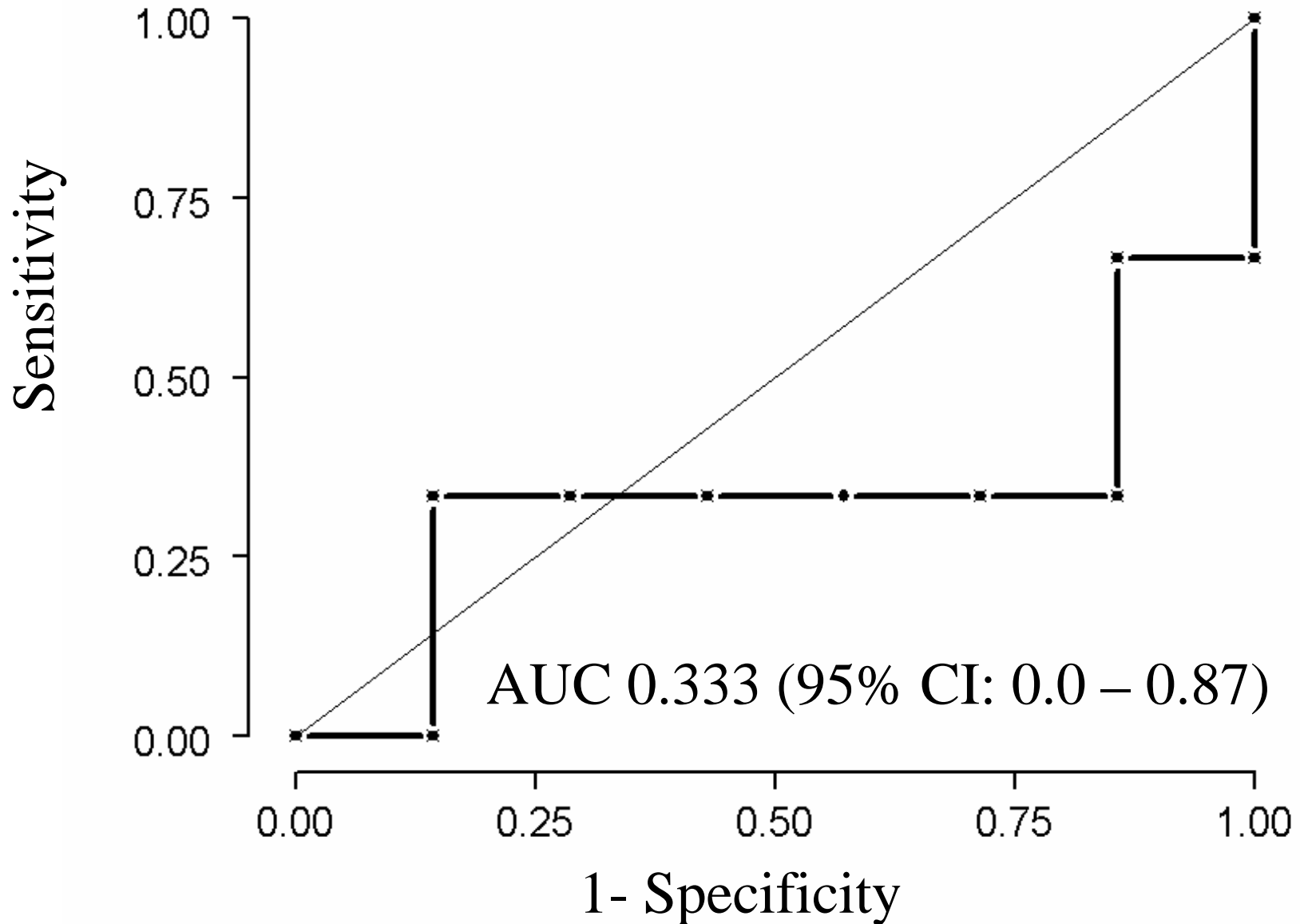# ROC for Glucose Stimulated Cytochrome c Reduction

AUC 0.889 (95% CI: 0.65 – 1.00)
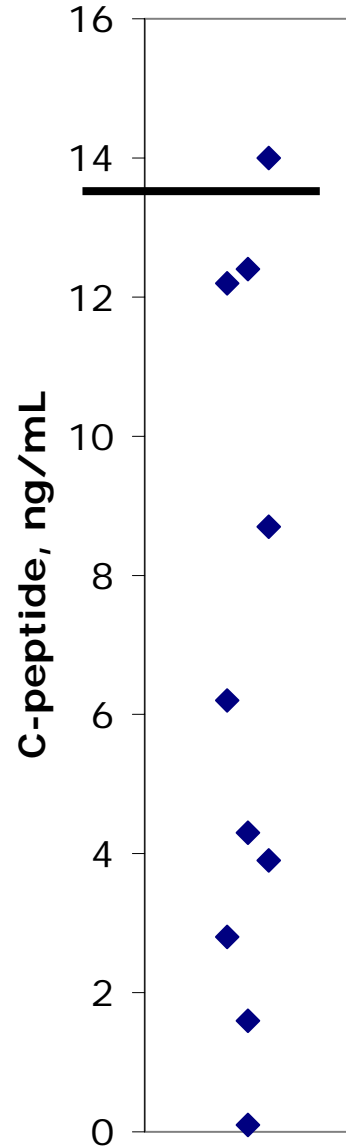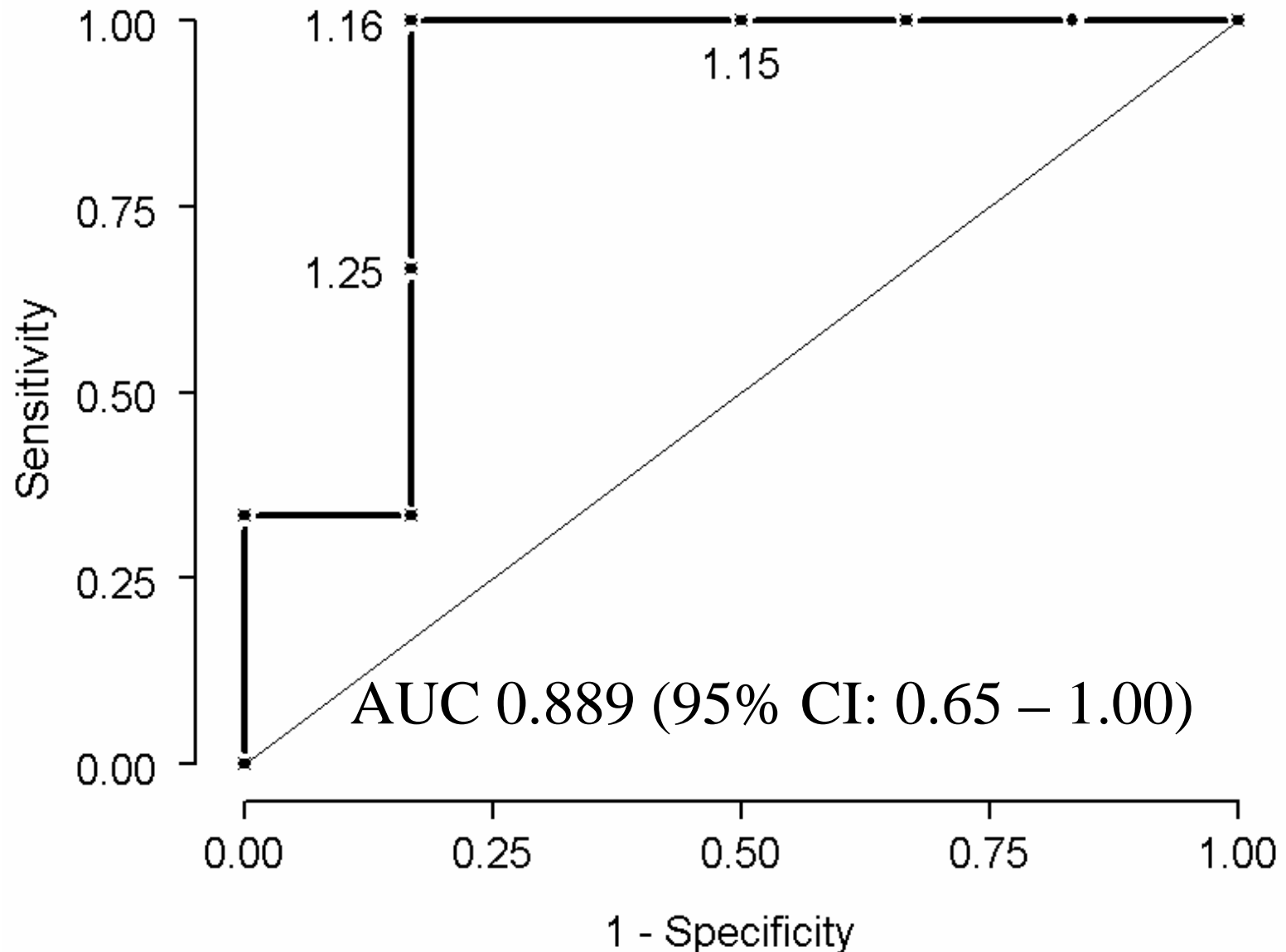
ROC for Glucose-Stimulated OCR

ROC for Oxygen Consumption at 3 mM Glucose

AUC 0.333 (95% CI: 0.0 – 0.87)

Summary

- Technical Features of Approach:
  - Reproducibility and reliability,
  - Non-invasive (multiple tests),
  - Continuous measurement over long periods of time .

- Validation
  - Detailed and quantitative studies on bioenergetics of islets confirmed that islets are well-maintained in the system and the assessment of the islets are highly reflective of the state of the islets.

- Using in vitro measurements of glucose-stimulated cytochrome c reduction and oxygen consumption, outcomes of in vivo secretory function by human islets transplanted into non-diabetic NOD-SCID mice was correctly predicted 88.9 and 80% respectively.

Conclusions

- The detection of glucose-stimulated cytochrome c reduction and oxygen consumption may have utility as criteria for the assessment of human islet quality.

1. Quick Turnaround:
    Assessment takes under 3 hours and data is online.
2. Potentially could be done with a single test.
3. Results are amenable to integrating assessment with total islet yield.

Acknowledgements

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