Published in final edited form as:

Transplant Proc. 2010 ; 42(6): 2020–2023. doi:10.1016/j.transproceed.2010.05.082.

Continuous Real-time Viability Assessment of Kidneys Based on Oxygen Consumption

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Abstract

Background—Current ex vivo quality assessment of donor kidneys is limited to vascular resistance measurements and histological analysis. New techniques for the assessment of organ quality before transplantation may further improve clinical outcomes while expanding the depleted deceased-donor pool. We propose the measurement of whole organ oxygen consumption rate (WOOCR) as a method to assess the quality of kidneys in real time before transplantation.

Methods—Five porcine kidneys were procured using a donation after cardiac death (DCD) model. The renal artery and renal vein were cannulated and the kidney connected to a custom-made hypothermic machine perfusion (HMP) system equipped with an inline oxygenator and fiber-optic oxygen sensors. Kidneys were perfused at 8°C, and the perfusion parameters and partial oxygen pressures (pO₂) were measured to calculate WOOCR.

Results—Without an inline oxygenator, the pO₂ of the perfusion solution at the arterial inlet and venous outlet diminished to near 0 within minutes. However, once adequate oxygenation was provided, a significant pO₂ difference was observed and used to calculate the WOOCR. The WOOCR was consistently measured from presumably healthy kidneys, and results suggest that it can be used to differentiate between healthy and purposely damaged organs.

Conclusions—Custom-made HMP systems equipped with an oxygenator and inline oxygen sensors can be applied for WOOCR measurements. We suggest that WOOCR is a promising approach for the real-time quality assessment of kidneys and other organs during preservation before transplantation.

Renal transplantation is the definitive therapy for patients with end-stage renal disease, offering increased longevity and improved quality of life.¹ At present, one of the main challenges in the field of transplantation is that demand for donor organs far exceeds the available supply. To address this disparity, the field has promoted programs for living donation, the use of organs from expanded-criteria donors, and donation after cardiac death (DCD).¹ As an increasing number of marginal organs are used, organ-quality assessment before transplantation has emerged as an important and urgent issue.

Current ex vivo quality assessment tools for donor kidneys are limited to histologic analysis² and measurement of vascular resistance during perfusion.³ The development of new real-time quantitative techniques for the assessment of organ quality ex vivo may potentially expand the

donor kidney pool. We present herein a technique for the continuous noninvasive measurement of whole organ oxygen consumption rate (WOOCR) as a method to assess the quality of organs during preservation and before transplantation. In addition to excluding organs that are unsuitable for transplantation, this method could allow for identification of healthy organs that would have otherwise been rejected as unsuitable.

Under normal physiologic conditions most viable tissues consume oxygen.⁴ Consequently, determining WOOCR could be useful for quantifying organ viability even when measurements are conducted during cold preservation (4–8°C). Calculation of oxygen consumption rate (OCR) has been applied before in the study of the heart,⁵ the liver,⁶ and the kidney.^{7–9} It has also been shown that OCR is a reliable and useful measure of pancreatic islet quality and can be used to predict islet transplant outcome.^{10–12}

We present WOOCR as a method for use with a modified hypothermic machine perfusion (HMP) system. HMP has been shown to be an effective method of organ preservation and is becoming a standard of clinical practice for kidney preservation among organ procurement organizations.¹³ Current clinical prototypes of HMP do not include oxygen sensors or an adequate oxygenation system to meet the oxygen demand of a viable kidney, even at cold temperatures. We propose 2 simple modifications to currently available clinical perfusion devices, including: 1) an inline oxygenator with oxygen supply; and 2) inline fiber-optic oxygen sensors, which are added to the HMP system without disturbing the organ. This setup provides the kidney with oxygenated perfusate and allows for real-time measurement of WOOCR.

MATERIALS AND METHODS

Porcine Kidney Procurement

Five porcine kidneys were procured from nonheart-beating donors (NHBDs). Each animal received an intravenous injection of 50,000–100,000 U heparin and was sacrificed using a lethal injection of pentobarbital sodium. Following exsanguination, the donor animals were eviscerated and the posterior wall of the aorta was incised longitudinally to expose the ostium of the renal artery (RA). RAs were individually cannulated, and each kidney was flushed with 1 L cold lactated Ringer solution (LRS) and carefully dissected away from the viscera. Warm ischemia time was minimized to <10 minutes during the procurement. Each kidney was weighed after procurement and before HMP.

Hypothermic Machine Perfusion and WOOCR Setup

Arterial and venous ends of each organ were cannulated and connected to the HMP system. In our custom-built HMP system, 2 fiber-optic oxygen sensors (Instech Labs, Plymouth Meeting, PA) were placed inline upstream of the RA and 2 placed downstream of the renal vein (RV) for the measurement of arterial and venous partial oxygen pressure (pO_2), respectively. To provide maximum oxygenation without causing vascular damage, the flow rate was controlled to be 100–200 mL/min and the temperature maintained at 8°C for the duration of the perfusion. The WOOCR of a kidney was calculated using a mass balance equation where Q is the flow rate of perfusion (mL/min), α is the solubility of oxygen at a given temperature (mol/mL · mm Hg), and m is the mass of the kidney (g).

$$OCR = \frac{Q \times (pO_{2_{Arterial}} - pO_{2_{Venous}}) \times \alpha}{m}$$

During perfusion of 4 kidneys, an oxygenator was incorporated into the setup to provide a steady supply of oxygenated preservation solution to the organ. Figure 1 shows the basic setup of the HMP system with inline fiber-optic oxygen sensors and the oxygenator.

Formalin Treatment of the Porcine Kidney

After a single porcine kidney was harvested and flushed with 1 L cold LRS, the initial measurement was performed to establish WOOCR before formalin treatment. Next, the kidney was flushed with 120 mL 10% buffered formalin through the RA, followed by a flush with 120 mL LRS to remove any remaining formalin. The new set of WOOCR data was collected 30 minutes after the formalin treatment.

RESULTS

As expected, without an oxygenator the measured pO_2 of the perfusate for both the RA and the RV approached zero within minutes while perfusing at 100 mL/min at 8°C. After incorporating an inline oxygenator, the inlet (RA) pO_2 was maintained at ~150 mm Hg (in equilibrium with room air), but the outlet (RV) pO_2 still remained near zero. When the arterial pO_2 was elevated to >300 mm Hg (using an oxygen supply), the outlet pO_2 increased and a significant pO_2 drop that was not limited by the oxygen supply was measured between the RA and RV for every kidney. The average WOOCR for presumably healthy kidneys on HMP with an inline oxygenator was 158 ± 62 nmol/min/g ($n = 4$; range 92–229 nmol/min/g). These results (and our theoretical calculations) verify the requirement of an inline oxygenator to provide an inlet pO_2 of >300 mm Hg for the adequate oxygenation of the entire organ and for the accurate measurement of WOOCR for the average porcine kidney. A dramatic drop in the WOOCR of the kidney after the formalin insult (17 nmol/min/g) was compared with the WOOCR of the same organ before formalin treatment (187 nmol/min/g; Fig 2). These results suggest that WOOCR can differentiate between damaged and healthy organs.

DISCUSSION

Current clinical HMP systems are not equipped with oxygenators. Rapid depletion of oxygen from the reservoir containing nonoxygenated preservation solution subjects the organ to hypoxic conditions. A theoretical calculation demonstrates that 1 L LRS equilibrated with air would be depleted of its oxygen content in ~5 minutes by an average “healthy” adult porcine kidney undergoing HMP. The experimental results confirm that arterial and venous pO_2 values of the recycled perfusate (ie, nonoxygenated) decreased to near zero after a few minutes of perfusion at 8°C. The results of this limited study clearly support the need for improved oxygen delivery in HMP systems during kidney preservation. One solution is to use an inline oxygenator which can supply a pO_2 of ≥ 300 mm Hg. Using a perfusion solution with a higher oxygen solubility could also improve oxygenation of tissue using a lower pO_2 .

With adequate oxygenation, a significant pO_2 drop that was not limited by the oxygen supply (ie, RV pO_2 was significantly >0) was observed between the RA and RV of every organ, and an average WOOCR value of presumably healthy kidneys on HMP was measured at 158 nmol/min/g. This value was similar to the OCR of healthy pancreatic tissue at 8°C (200 nmol/min/g).¹⁰

Our results also indicated a significant variability in WOOCR among freshly procured kidneys (range 92–229 nmol/min/g) that were harvested using our standardized technique. We hypothesize that the variability in WOOCR among these organ can be correlated with the individual organ quality and may identify damaged organs ex vivo that otherwise would be undetected.

The difference between the WOOCR in presumably healthy and damaged organs was supported by comparing the WOOCR of a formalin-treated (damaged) kidney (17 nmol/min/g) to the WOOCR of the same organ before treatment (187 nmol/min/g) or the average WOOCR of all 4 untreated organs (158 nmol/min/g). Detection of any WOOCR in the formalin-treated kidney suggests the incomplete damage of the organ and reflects the OCR of the remaining viable tissue. The WOOCR of the kidney would be expected to approach zero if the amount of fixate and the duration of the fixation were increased.

Based on these data, we conclude that a real-time noninvasive method for the measurement of WOOCR in conjunction with oxygenated HMP may be used to assess organ quality *ex vivo*. These measurements are feasible using a modified clinical prototype of HMP system.

The role of oxygenation in organ preservation remains controversial.^{6,14-15} The optimum HMP preservation will need to balance the potential damage that can occur with the generation of free radicals with the necessity to provide sufficient oxygen to sustain healthy tissues. Our future research will be directed toward further validation of the WOOCR method by correlating measurements to histologic data, molecular profiles, and graft function after allogeneic transplantation in a large animal model.

Acknowledgments

The authors thank Mike Taylor, Simona Baicu, Mike Loughnane, Kristen Maynard, Brian Perrault, Mel Graham, Luke Mutch, Takayuki Anazawa, Ichiro Matsumoto, and Ron Joki for administrative and scientific support.

Supported by grants from the National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases (R43 DK070400), the Schott Foundation, the Carol Olson Memorial Diabetes Research Fund, the United Metal Traders, the Eunice L. Dwan Trust, the Iacocca Family Foundation, and Tom and Meredith Olson.

References

1. Knoll G. Trends in kidney transplantation over the past decade. *Drugs* 2008;68(Suppl 1):3–10. [PubMed: 18442296]
2. Reznik ON, Bagnenko SF, Loginov IV, et al. Machine perfusion as a tool to select kidneys recovered from uncontrolled donors after cardiac death. *Transplant Proc* 2008;40:1023. [PubMed: 18555105]
3. Snoeijs MGJ, Buurman WA, Christiaans MHL, et al. Histological assessment of preimplantation biopsies may improve selection of kidneys from old donors after cardiac death. *Am J Transplant* 2008;8:1844. [PubMed: 18786228]
4. Papas KK, Hering BJ, Guenther L, et al. Pancreas oxygenation is limited during preservation with the two-layer method. *Transplant Proc* 2005;37:3501. [PubMed: 16298642]
5. Stowe DF, Camara AK, Heisner JS, et al. Low-flow perfusion of guinea pig isolated hearts with 26° C air-saturated Lifer solution for 20 hours preserves function and metabolism. *J Heart Lung Transplant* 2008;27:1008. [PubMed: 18765194]
6. Dutkowski P, Furrer K, Tian Y, et al. Novel short-term hypothermic oxygenated perfusion (HOPE) system prevents injury in rat liver graft from non-heart beating donor. *Ann Surg* 2006;244:968. [PubMed: 17122622]
7. Levy MN. Oxygen consumption and blood flow in the hypothermic, perfused kidney. *Am J Physiol* 1959;197:1111. [PubMed: 14416432]
8. Stubenitsky BM, Booster MM, Brasile L, et al. Ex vivo viability testing of kidneys after postmortem warm ischemia. *ASAIO J* 2000;46:62. [PubMed: 10667719]
9. Crane NJ, Pinto PA, Hale D, et al. Non-invasive monitoring of tissue oxygenation during laparoscopic donor nephrectomy. *BMC Surg* 2008;8:8. [PubMed: 18419819]
10. Papas KK, Pisanía A, Wu H, et al. A stirred microchamber for oxygen consumption rate measurements with pancreatic islets. *Biotechnol Bioeng* 2007;98:1071. [PubMed: 17497731]

11. Papas KK, Colton CK, Nelson RA, et al. Human islet oxygen consumption rate and DNA measurements predict diabetes reversal in nude mice. *Am J Transplant* 2007;7:707. [PubMed: 17229069]
12. Papas KK, Suszynski TM, Colton CK. Islet assessment for transplantation. *Curr Opin Org Transplant* 2009;14:674.
13. Taylor MJ, Baicu SC. Current state of hypothermic machine perfusion preservation of organs: The clinical perspective. *Cryobiology*. Oct 24;2009 Published online.
14. Kim J, Seok YM, Jung KJ, et al. Reactive oxygen species/oxidative stress contributes to progression of kidney fibrosis following transient ischemic injury in mice. *Am J Physiol Renal Physiol* 2009;297:F461. [PubMed: 19458120]
15. Treckmann J, Nagelschmidt M, Minor T, et al. Function and quality of kidneys after cold storage, machine perfusion, or retrograde oxygen prsufflation: results from a porcine autotransplantation model. *Cryobiology* 2009;59:19. [PubMed: 19345683]

Fig 1.
Simple OCR perfusion system schematic, illustrating the perfusion circuit containing the inline oxygenator and oxygen sensors.

Fig 2.
WOOCR measurements from a single kidney immediately after the procurement and 30 minutes after formalin flush.