

Continuous Measurement of Oxygen Consumption by Pancreatic Islets

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ABSTRACT

The rate of oxygen consumption is an important measure of mitochondrial function in all aerobic cells. In pancreatic beta cells, it is linked to the transduction mechanism that mediates glucose-stimulated insulin secretion. However, measurement of oxygen consumption over long periods of time is technically difficult owing to the error resulting from baseline drift and the challenge of measuring small changes in oxygen tension. We have adapted an ultrastable oxygen sensor based on the detection of the decay of the phosphorescent emission from an oxygen-sensitive dye to a previously developed islet flow culture system. The drift of the sensor is approximately 0.3%/24 h, allowing for the continuous measurement of oxygen consumption by 300 islets (or about 6×10^5 cells) for hours or days. Rat islets placed in the perfusion chamber for 24 h were well maintained as reflected by membrane integrity, insulin secretion, and oxygen consumption. Both acute changes in oxygen consumption as induced by glucose and chronic changes as induced by sequential pulses of azide were resolved. The features of the flow culture system—aseptic conditions, fine temporal control of the composition of the media, and the collection of outflow fractions for measurement of insulin, and other products—facilitate a systematic approach to assessing metabolic and functional viability in responses to a variety of stimuli. Applications to the measurement of effects of hypoxia on insulin secretion, membrane integrity, and the redox state of cytochromes are demonstrated. The system has particular application to the field of human islet transplantation, where assessment and the study of islet viability have been hampered by a lack of experimental methods.

INTRODUCTION

MITOCHONDRIAL METABOLISM plays a dual role in the pancreatic beta cell, both (a) in the provision of energy needed to maintain cellular function and (b) as a mediator of glucose induced insulin secretion.¹ Despite the importance of oxygen consumption to these processes, its study has been hampered by the technical

difficulties of measuring small changes in oxygen tension. The measurement of oxygen consumption in a flow system is based on the difference between the inflow and outflow oxygen tensions. Since islets are particularly sensitive to hypoxia, the outflow oxygen tension should not be allowed to drop to levels that will impair viability. The standard method for detecting oxygen tension, the Clark electrode, is not stable

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over time (baseline drift is greater than 1%/h), making its use for chronic assessment of oxygen consumption impractical.^{2,3} To prevent hypoxia from impairing islet viability when using gas equilibrated with air (142 mm Hg), the difference between inflow and outflow oxygen tension cannot be greater than about 20%. Therefore, even a drift of 0.5%/h will yield a measurement error of 60% over 24 h. Thus, studies of oxygen consumption by islets using the continuously stirred chamber,³ perfusion analysis,² and a microelectrode placed into the islet⁴ have been limited to 1–2 h. The duration of experiments using closed systems, including the Cartesian diver,^{5–7} and the continuously stirred chamber is limited by the oxygen content of the solution. Moreover, throughout the experiment the islets are subjected to increasing levels of hypoxia. To circumvent these problems, an ultra-stable oxygen sensor based on the detection of the decay of the phosphorescent emission from an oxygen-sensitive dye^{8,9} was applied to a previously developed islet flow culture system.¹⁰ Both acute and chronic changes in oxygen consumption can be continuously monitored, making it particularly suited to the study of slow processes such as the progression of apoptosis and necrosis. The flow culture system allows for precise control over aqueous and gaseous composition of the inflow and can be run aseptically for hours or days. Very small differences between inflow and outflow oxygen tension can be robustly measured, thereby avoiding exposure of the islets to hypoxia. In traditional fashion, the outflow from the perfusion chamber can be collected and assayed for insulin and other hormones and metabolites. This approach may be particularly important in the study of human islet transplantation, where optimization in the preparation and culture of islets has been hampered by means of assessing viability and a lack of understanding of the mechanisms of cell death.^{11,12}

MATERIALS AND METHODS

Chemicals

The medium used for islet perfusions was RPMI medium 1640 (supplied without glucose by Gibco BRL, Grand Island, NY) supple-

mented with 10% (vol/vol) fetal bovine serum (FBS), antibiotic–antimycotic (100 U/ml penicillin, 100 μ g/ml streptomycin, and 0.25 μ g/ml amphotericin B), 2 mM glutamine (all purchased from Gibco BRL), and varying amounts of glucose (Sigma Chemical, St. Louis, MO). Perifusions of INS-1 cells were done with Krebs–Ringer bicarbonate solution containing 20 mM HEPES (Roche Molecular Biochemicals, Indianapolis, IN), 98.5 mM NaCl (Fisher, Houston, TX), 4.9 mM KCl, 1.2 mM potassium phosphate, 1.2 mM magnesium sulfate, 2.6 mM calcium chloride, and 25.9 mM sodium bicarbonate (all purchased from Mallinckrodt Baker, Inc., Phillipsburg, NJ), 0.1% CRG-7 albumin (Intergen Co., Purchase, NY), and varying amounts of glucose. Cytochrome c, sodium dithionite, potassium ferricyanide, sodium azide, acridine orange, and propidium iodide were all purchased from Sigma-Aldrich (St. Louis), and cytochrome oxidase was prepared by the method of Yonetani.¹³

Islet isolation

Rat islets from male BB rats (approximately 200 g) were harvested after collagenase-mediated pancreas digestion using Liberase™ (Roche Molecular Biochemicals), which was injected into the pancreatic duct and used per the manufacturer's instructions. Islets were then purified in a gradient solution of Optiprep™ (Nycomed, Olso, Norway).¹⁴

Membrane integrity of islets

Viability of islets was calculated after staining an aliquot of islets with acridine orange (10 μ mol/L) and propidium iodide (15 μ mol/L).¹⁵ Islets were visually inspected using an inverted fluorescence microscope (TE200 with a 10 \times objective, Nikon, Melville, NY), and individual islets were given a score of 0%, 25%, 50%, 75%, or 100% viable according to the percentage of the cells of the islet stained green versus red. Total viability for the group of islets was calculated as the average of these scores.

INS-1 cell culture

INS-1 cells were grown in RPMI medium 1640 containing 2 mM glutamine, 1 mM pyru-

vate, 10% (vol/vol) FBS, and 1 mM β -mercaptoethanol (Sigma Chemical). The day before the perfusion, INS-1 cells were detached by washing and incubating with phosphate-buffered saline for 20–40 min, followed by dislodging cells under a gentle stream of flowing media from a transfer pipette (5 mL, Samco Scientific Corp., San Fernando, CA). The cells were then incubated overnight in non-tissue culture petri dishes containing RPMI medium 1640 prepared as described above.

Perfusion system

The perfusion system was similar to that previously described¹⁰ and consisted of (1) a multichannel peristaltic pump (MiniPuls 2, Gilson, Middleton, WI) and 0.25 mm i.d. tubing (Elkay Products, Inc., Shrewsbury MA), (2) a Rheodyne sample injector (model 7725, Rheodyne L.P., Rohnert Park, CA) for infusing

test agents, (3) a gas exchanger in which media flowed through 4 in. of thin-walled Silastic™ tubing (0.062 in. i.d. \times 0.095 in., Dow Corning Corp., Midland, MI) loosely coiled in a 125-mL glass jar that contained 5% CO₂/balance air or as indicated, (4) a perfusion chamber (Fig. 1), and (5) a Foxy 200 fraction collector (Isco, Inc., Lincoln, NE). Both the gas exchanger and the perfusion chamber were immersed in a 37°C water bath. Gas was supplied to the gas exchanger at a flow rate of 1–2 mL/min, and the jars contained approximately 5 mL of water to humidify the gas. All media vessels were sterilized by autoclave. Perfusion columns and tubing systems were gas-sterilized with ethylene oxide.

Perfusion column

The perfusion column consisted of a 4-mm i.d. \times 2-cm-long glass tube into which a cus-

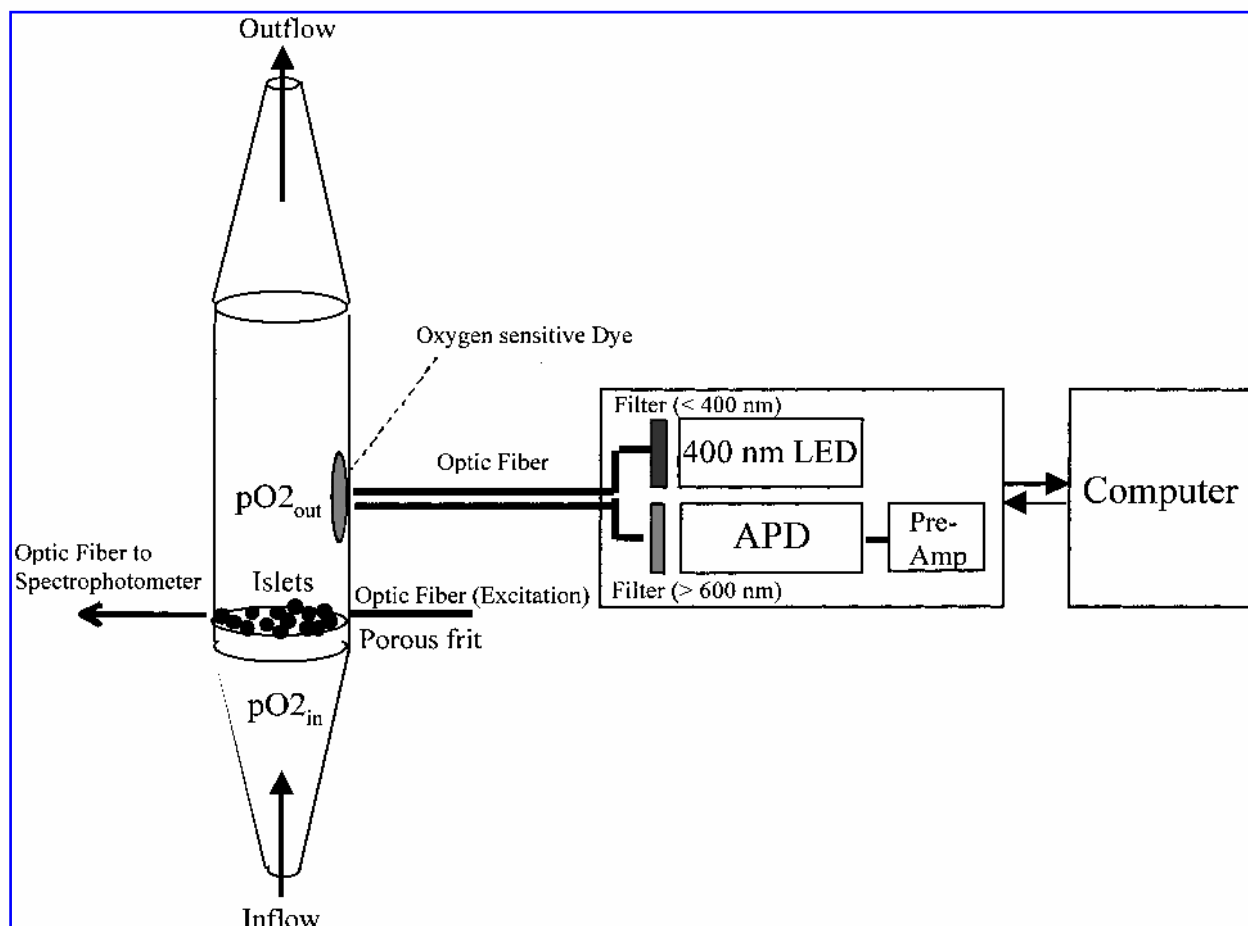


FIG. 1. Perfusion chamber and optical detection of oxygen consumption and cytochromes.

tom-made polyethylene frit (4 mm o.d. \times 1 mm thick) was inserted (Fig. 1). The frit was easily pushed into the lumen of the tube, but did not move owing to the pressure of the slow flow rates. Conical glass tubing was affixed onto both ends of the tubing (10 mm long \times 1 mm i.d. at the end of the tube). The islets rested on the frit, while media emanated from beneath them. Dye injection studies confirmed that the flow front was uniform across the width of the chamber (data not shown).

Loading the cells and islets into the columns

Prior to adding the cells or islets to the chamber, medium was pumped through the system for 60 min. All additions to the columns were done inside a BioGard Class II Type A Hood (Baker Co., Inc., Sanford, ME) to maintain sterility. Islets were loaded into the chamber as follows. Islets were handpicked using a P200 Pipetman (Gilson) into a 0.6-mL microfuge tube and allowed to settle. With the pump off, the outflow tube was disconnected from the chamber, and 100 μ l of media was removed from the perfusion chamber. Islets were transferred from the microfuge tube to the bottom of the perfusion chamber using a P200 pipette and a gel-loading tip (Island Scientific, Bainbridge Island, WA). Islets were allowed to settle for 1 min before replacing the outflow chamber and starting the flow. Recovery of islets was accomplished by removing the outflow tube, inverting the column, and pouring the contents of the chamber into a petri dish. Islet recoveries were typically greater than 95%.

For the INS-1 cell perfusion, the cell suspension was transferred to a centrifuge tube with a plastic serological pipette. The tube was centrifuged at 600 *g* for 3 min, and the supernatant was aspirated until there was 100 μ L remaining. The pellet was triturated with a P100 pipette and loaded in the same manner as for islets.

Oxygen detection system

Oxygen tension was measured on line by phosphorescence lifetime detection of an oxygen-sensitive platinum porphyrin, which was painted onto the inside of the perfusion chamber 3 mm above the islets (Fig. 1). The dye mol-

ecule was a platinum tetrapentafluorophenyl porphyrin and polycarbonate-silicone copolymer¹⁶ and was made by dissolving 5 g of a dimethylsiloxane-bisphenol A-polycarbonate blockcopolymer (GE, Waterford, NY) in 100 mL of dichloromethane and 25 mg of platinum tetrapentafluorophenyl porphyrin (Porphyrin Products, Logan, UT). About 0.1 mL of the dye solution was dispensed onto the inner surface of the perfusion chamber and left to air-dry. A thin film formed that was interrogated noninvasively from the outer wall, thereby assessing the oxygen content of the medium after it has perfused the islets. The dye molecules were excited with a 50 μ s pulse of light (395 nm) from a UV LED (HUUV-5102, Roithner, Vienna, Austria), after which they emit a phosphorescent red light with a characteristic emission decay lifetime of 20–100 μ s. Excitation and detection of phosphorescence were done using a lifetime detection apparatus⁸ via an optical fiber bundle. The lifetime of the emission was quantified by comparing the amount of light emitted from 0 to 30 μ s after the excitation pulse (I_a) with that emitted from 30 to 150 μ s after the excitation pulse (I_b). The ratio of intensity over the two periods, $R = I_b/I_a$, is the output of the instrument and increases with decreasing oxygen concentration. Sensor films were calibrated by equilibrating known premixed oxygen/5% carbon dioxide/balance nitrogen gas with flowing media in the flow culture system. Oxygen consumption was calculated as the flow rate times the difference in oxygen tension between the inflow and outflow. The inflow oxygen tension was measured in the absence of islets in the chamber both before and after each experiment, and for the calculations, oxygen content of media equilibrated with 5% carbon dioxide/balance air was assumed to be 217 nmol/mL.¹⁷

Detection of cytochromes

Cytochromes were analyzed by transmission of light from a constant intensity quartz-tungsten-halogen white light source (model 66184, Oriel Instruments, Stratford, CT) through the column of cells in the chamber (Fig. 1). Prior to entering the column, the light beam passed through a 1-in. water filter and electromechan-

ical shutter (model 76995, Oriel Instruments). Constant intensity was assured by a photofeed-back system (model 68850, Oriel Instruments), to adjust for drift over time. Spectra from 450 to 950 nm were acquired via a diffraction spectrograph (model 100S, American Holographics, Littleton, MA), on a 512-pixel photodiode array (model C4350, Hamamatsu, Bridgewater, NJ), converted into digital form using a 16-bit analog-to-digital converter (model AT-MIO-16X, National Instruments Austin, TX), and entered into a separate desktop computer (Gateway2000 66MHz, Gateway, North Sioux City, SD). Spectra were acquired every 8 s with an integration time of 50 ms and referenced to a 2% Intralipid solution (Baxter, Deerfield, IL). Second derivatives of absorbance with respect to wavelength were determined using a computer algorithm written in Matlab (The Mathworks, Natick, MA).

Insulin assay

Insulin was measured by enzyme-linked immunosorbent assay per instructions supplied by the manufacturer (ALPCO, Windham, NH).

RESULTS

Overview of system

The flow culture system used was similar to a previously developed system¹⁰ but was modified in order to accommodate low flow rates and small numbers of islets. A peristaltic pump delivered perfusate to a gas exchanger, which equilibrated the medium with a gas mixture of 5% carbon dioxide and the desired oxygen content. A Rheodyne sample injector was placed in between the pump and the gas exchanger to facilitate rapid changes of medium composition. The sample loop in the sample injector held up to 3 mL, which would allow for a 60-min square wave change in composition at 50 μ L/min; the sample loop can be manually refilled, and the contents reinjected, allowing for changes in composition that could last for several hours. From the gas exchanger, the perfusate traveled through the inlet port and frit to disperse the flow and homogeneously bathed the islets in the perfusion chamber. The

glass chamber allowed for optical interrogation of the contents of the chamber: optical fiber probes for oxygen tension, and cytochromes (absorbance spectra) were held in place by a plexiglass block with set screws (Fig. 1). The

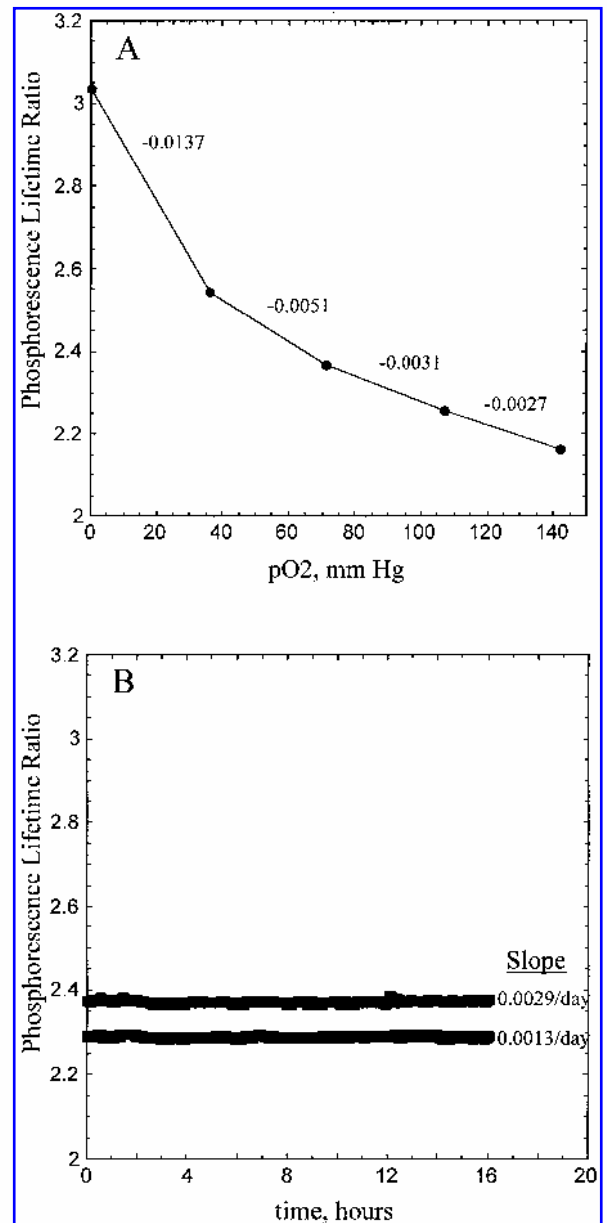


FIG. 2. Oxygen sensor calibration curve and baseline characterization. **A:** The effect of oxygen tension in the perfusion medium on the phosphorescent ratio was determined after equilibrating the inflow with gases containing 0%, 5%, 10%, 15%, and 20% oxygen. Numbers are linearized slopes between calibration points. **B:** Stability of phosphorescent ratio over 15 h. The flow culture system was run in the absence of islets in the chamber, where the medium was equilibrated with 5% CO₂/95% air. Drift corresponded to 0.3%/day, and the coefficient of variation of all measurements during the 15 h was 0.6%.

perfusion chamber and optical probes were submerged in the 37°C water bath during experiments.

Characteristics of oxygen detection system

The lifetime detection algorithm involves a 50- μ s excitation pulse, followed by detection of the emitted phosphorescence from 0 to 30 μ s and 30 to 150 μ s. The ratio of these two signals ($R = I_b/I_a$) is a nonlinear function of the oxygen tension in the media (Fig. 2A), whose linearized slope between each calibration point was extremely stable from experiment to experiment. In practice, the slopes were determined, and then for each experiment only a single point was needed to calibrate the system (typically 20% oxygen was used).

Measuring the decay rate of emission rather than only the intensity produces a more robust sensor whereby several problems that arise with intensity measurement are avoided such as light source intensity fluctuations and vibrations in film thickness and dye concentration. To validate the stability of the oxygen sensor, a flow culture experiment was done in the absence of islets in the chamber, and the oxygen lifetime ratio was plotted (Fig. 2B). Fitting the data with a straight line yielded that the drift of the oxygen detection system was 0.3%/day, and the coefficient of variation of all the measurements over a 15-h period (at one

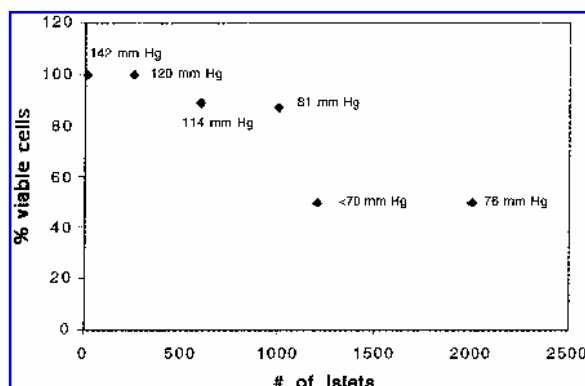


FIG. 3. Effect of islet density on islet cell viability. Varying numbers of islets (10, 300, 600, 1,000, 1,200, and 2,000) were perfused for 24 h at 50 μ L/min. Also shown is the lowest oxygen in the outflow observed over the 24-h period. At islet numbers of 300 or fewer the islets in the perfusion system retained membrane integrity and experienced minimal hypoxia.

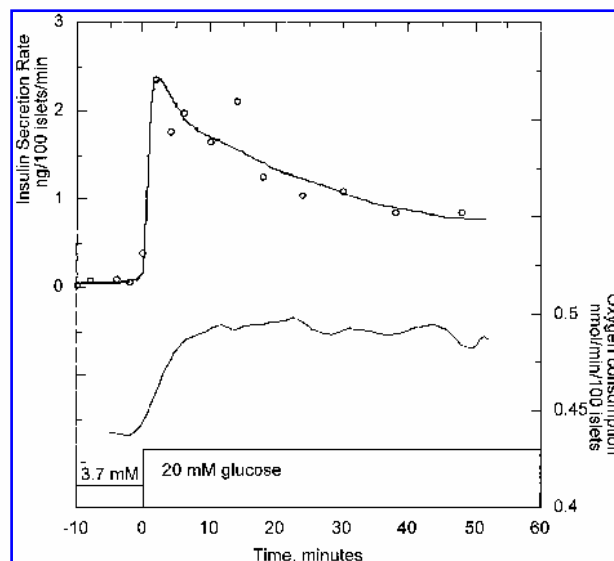


FIG. 4. Acute responses in insulin secretion and oxygen consumption to glucose. Shown is the effect of a step change in glucose on insulin secretion and oxygen consumption, 3 h after loading islets into chamber. The concentration of glucose was 3.7 mM for the first 3 h followed by 1 h at 20 mM.

per 2 min) was 0.6%. To obtain a signal-to-noise ratio of 10:1, this means that a 6% difference in the inflow and outflow oxygen tension (equivalent to a change of 8.5 mm Hg) is needed for robust detection of oxygen consumption.

Viability of densely packed islets in the chamber

The membrane integrity of islets was determined after placing varying numbers of islets (10–2,000) in the chamber and perfusing for 24 h at a flow rate of 50 μ L/min. Oxygen tension was measured continuously; at the end of this time the islets were removed from the chambers, and the percentage of the islets that stained with propidium iodide was quantified (Fig. 3). Placing 600 islets or more in the chamber resulted in a loss of membrane integrity, presumably due to the resulting hypoxia: The oxygen tension in the outflow decreased as the number of islets placed in the chamber increased. Three hundred islets produced a change on the order of 20 mm Hg in oxygen tension, which represents a signal-to-noise ratio of about 25. Assuming that islets were 150 μ m in diameter and formed a monolayer in the chamber, then about 680 islets would fully occupy the surface area on the frit. Thus, between

300 and 600 islets represents a transition where space between islets increases, which may be a factor for the maintenance of islet viability.

Acute response to metabolic stimulus

Characterizing the dynamic response time of the system directly is not easily obtainable owing to the difficulty in producing a precisely defined change in oxygen tension in the input. The oxygen sensor has a response time on the order of a few seconds (data not shown). However, the response time of the oxygen detector incorporated into the flow culture system will include the delay and dispersion of the oxygen as it flows and diffuses from the media surrounding the islets to the oxygen sensor. In order to demonstrate the ability of the oxygen detection system to track acute changes in oxygen consumption, islets were subjected to a step increase in glucose concentration. Classical biphasic response in insulin secretion was observed, demonstrating normal functional integrity (Fig. 4). Concomitantly, oxygen consumption increased, reaching a plateau within 5 min of the start of the change in glucose. Since some of this equilibration time was due to response time of the islets, the response time of the oxygen detection system is on the order of 2–4 min. These acute results are similar to those obtained by previous studies.^{2,18}

Chronic changes in islet oxygen consumption tracked by the system

A unique feature of the system is the ability to continuously assess oxygen consumption over hours or days, allowing for the temporal characterization of processes that are kinetically slow. To test the ability of the system to continuously assess changes in oxygen consumption over extended periods of time, islets in the system were subjected to 30-min pulses of 3 mM azide every 2 h (Fig. 5). Oxygen consumption decreased 30–40% in response to the azide and then rapidly returned to baseline after the azide washed out. In the absence of changes in the composition of the media, oxygen consumption was very stable over 20 h, indicating that islets are well maintained in the perfusion system. Thus, changes in mitochondrial respiratory rate can be characterized in

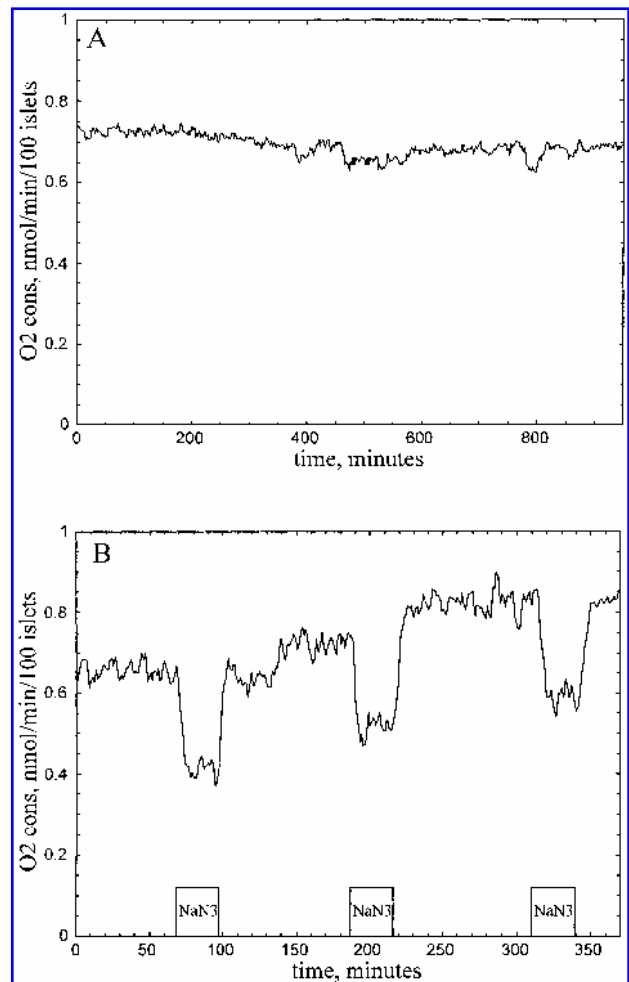


FIG. 5. Chronic measurement of oxygen consumption by islets in the flow culture system. **A:** Oxygen consumption by 300 islets over a 15-h period was stable, demonstrating that the detection methodology is robust and the islet viability is unchanged. **B:** Continuous assessment of kinetic changes in islet oxygen consumption induced by the pulsatile administration of a reversible inhibitor of cytochrome c (3 mM sodium azide). Three hundred rat islets were perfused for 8 h and continuously assessed for oxygen consumption, during which time azide was infused for 30 min every 2 h. RPMI medium 1640 containing 10% FBS and 11.1 mM glucose was pumped at a flow rate of 50 μ L/min. Stability of oxygen consumption between pulses and time to reach a new plateau level at the beginning and the end of each pulse demonstrates the system's ability to track acute and chronic changes in oxygen consumption.

the flow culture over the course of hours or days.

Control of inflow gas composition

The gas equilibration system allows for the kinetic response of changes in dissolved gases to be assessed. As an illustrative example of the

utility of this feature, oxygen tension in the media was altered. When supplying the lung with 5% CO₂/95% air, islets responded to an increase in glucose concentration by secreting insulin in a biphasic manner (Fig. 6). In contrast, insulin release by islets perfused with media equilibrated with 5% CO₂/95% nitrogen was not detectable. These results indicate that the time course of the functional and respiratory response to, and recovery from, alterations in the gas composition may be examined with the flow culture system.

Continuous measurement of redox state of cytochrome c and cytochrome oxidase

To demonstrate the feasibility of measuring cytochromes, INS-1 cells were loaded into the chamber, and transmission spectra were acquired from 450 to 950 nm. Valleys were observed in the second derivative spectra that corresponded to cytochrome c (550 nm) and cytochrome oxidase (605 nm) (Fig. 7A). The valleys were sensitive to changes in oxygen tension, reflected in a time-dependent flattening of the second derivative of the spectrum at increasing oxygen levels (Fig. 7B). As expected,^{19,20} the kinetic response of cytochrome oxidase was similar to that of cytochrome c (data not shown), indicating that the two steps maintain near-equilibrium status. These results demonstrate that changes in the reduction state of cytochromes *in situ* in cells can be continu-

ously and noninvasively detected in the flow culture system.

DISCUSSION

Development of a microflow culture system to maintain and assess islets

In a previous article, we reported the features of a flow culture system designed to maintain and assess large amounts of islet tissue.¹⁰ We have modified the system to be applied to small amounts of islets or cells (300–600 × 10³ cells) and have adapted two optical methods to measure oxygen consumption and cytochrome reduction of the islets. The ability of the flow culture system to control the inflow composition and operate aseptically provides a powerful platform for both acute and chronic studies. In addition, by collecting fractions, kinetic profiles of the release of insulin can be measured to relate mitochondrial function with insulin secretion.

Measurement of oxygen tension

In order to continuously assess oxygen consumption in our flow culture system, a sensor has been developed utilizing a Pt-porphyrin dye, whose decay of phosphorescence is inversely related to the oxygen tension.^{8,16} The use of this type of sensor for biological systems was first reported by Vanderkooi and Wilson,²¹

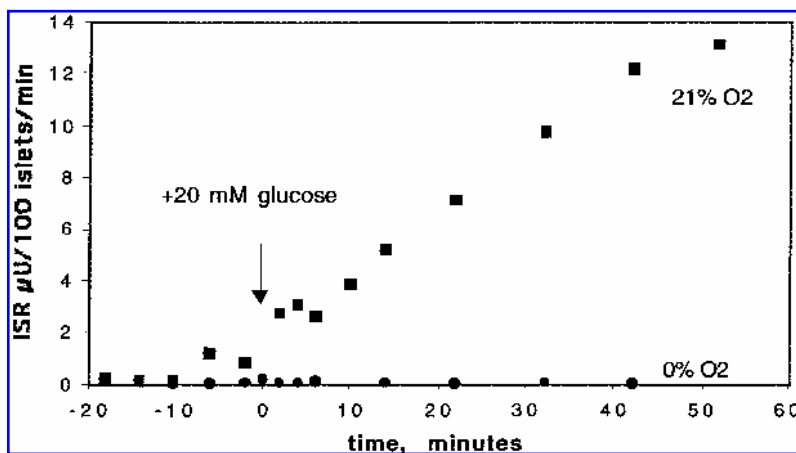


FIG. 6. Effect of hypoxia on glucose-induced insulin secretion rate by rat islets in the flow culture system. Rat islets (150 per chamber) were perfused for 2 h with RPMI medium 1640 (containing 10% FBS and 3 mM glucose) equilibrated with either 5% CO₂/balance air or 5% CO₂/balance nitrogen. A step change in glucose induced biphasic secretion of insulin by the islets perfused with normoxic media. In contrast, insulin secretion by islets exposed to anoxic media was not detectable.

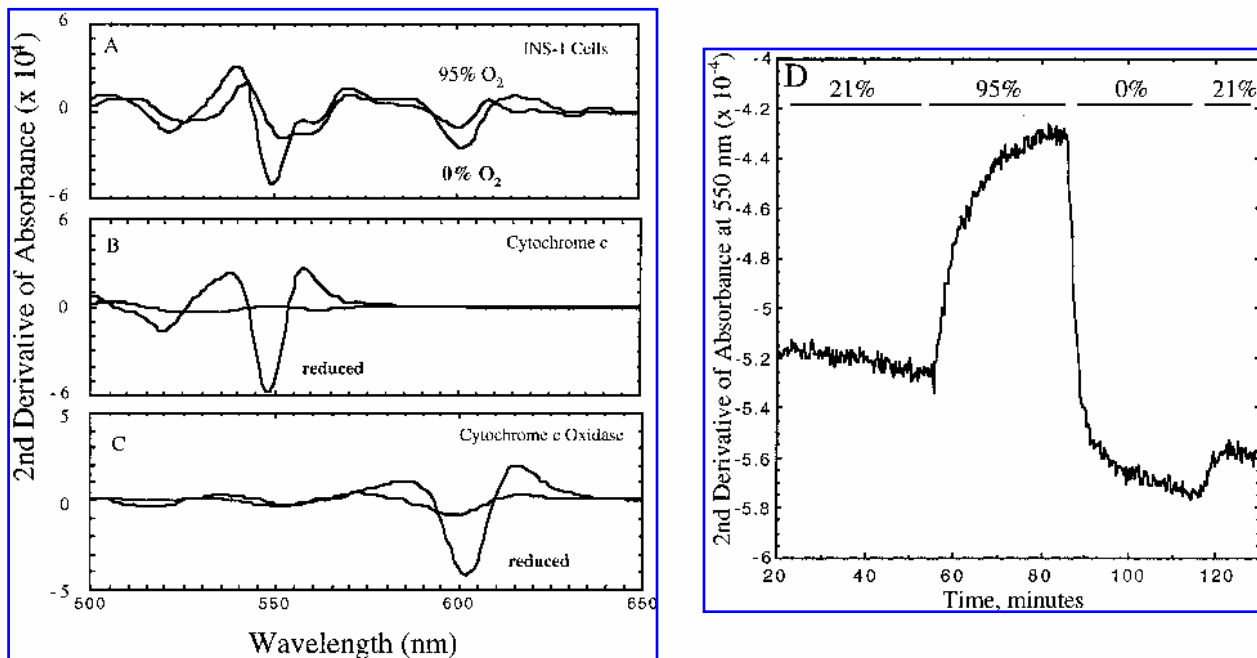


FIG. 7. Effect of oxygen tension on redox state of cytochromes in perfused INS-1 cells. **B** and **C**: Reference spectra of purified cytochrome c and cytochrome oxidase (approximately $50 \mu\text{M}$). In the reduced state (in the presence of an excess of sodium dithionite), second derivatives of absorbance spectra are characterized by valleys centered around 550 nm for cytochrome c and 605 nm for cytochrome oxidase. In contrast, when the cytochromes are oxidized (in the presence of an excess of potassium ferricyanide), their spectra become flatter. **A**: Second derivative spectra obtained from transmittance of white light through the perfusion column containing INS-1 cells and perfused with buffer equilibrated with either 95% or 0% oxygen. The valleys centered around 550 and 605 nm flatten out when the cells are bathed in buffer containing high oxygen tension, reflecting an increase in the oxidation state of the cytochromes. **D**: Time course of the experiment shown by the second derivative of absorbance at 550 nm. The magnitude of the second derivative increased with dissolved oxygen with a time constant on the order of 5 min. Changes at 605 nm were kinetically indistinguishable from those seen at 550 nm (data not shown).

who used a Pd-porphyrin dye, whose stability and applicability have been described.²² The remarkable stability of the sensor derives from the principle of lifetime detection, which is based on the ratio of initial phosphorescence intensity to the final, thereby canceling out any drift in detector efficiency and signal acquisition. Furthermore, the speed at which the lifetime measurement can be made allows for the averaging of 20,000 excitation/detection cycles per measurement (at one measurement per minute). The baseline drift of the oxygen detection system was only 0.3%/day (Fig. 2), which is about 100 times more stable than the Clark electrode.

Measurement of oxygen consumption in the flow culture system

Measurement of oxygen consumption in a flow system requires that islets in the perfusion chamber remove enough oxygen from the

perfusate so that the outflow and inflow media contain a detectable difference in oxygen tension. However, the oxygen depletion cannot be so great as to induce hypoxic effects on islet viability. We determined that at a flow rate of $50 \mu\text{L}/\text{min}$, 300 islets produced a minimum outflow oxygen tension of 110 mm Hg (Fig. 3). After 24 h in the perfusion system, no loss of membrane integrity was seen, and oxygen consumption remained constant during this period (Fig. 5), indicating that oxygen consumption by islets can be continuously monitored without loss of viability. In addition to maintenance of islet viability and the stable detection of oxygen tension, another prerequisite for the chronic assessment of islet oxygen consumption is a short dynamic response time of the detection system. Oxygen consumption responded rapidly to increases in glucose concentration, reaching a new equilibrium value within 5 min (Fig. 4), reflecting a system response time of 2–4 min. For increased kinetic

resolution, flow rates and numbers of islets could be increased (or the chamber could be made smaller), but this degree of resolution was adequate for chronic assessment over hours and days. Most importantly, we have demonstrated that the expected increases and decreases in oxygen consumption after addition and removal of an inhibitor of cytochrome *c* were precisely tracked by the oxygen detection system (Fig. 5). The ability to track oxygen consumption over many hours or days allows the system to be able to characterize the kinetics of slow processes such as apoptosis and the effects of culture conditions on viability.

In addition to the robustness of the oxygen detection system, additional features of the system are the ability to implement precise changes in aqueous and gaseous composition of the media and to make simultaneous measurements of other metabolic and functional correlates that complement the oxygen consumption data. In a preliminary test of the system, insulin secretion was fully inhibited by perfusing islets with anoxic media (Fig. 6). The use of the gas equilibration system can be used to characterize the acute and chronic kinetic responses to changes in gas composition. In general the effect of gases on islets has been limited to static conditions. A previous perfusion system developed by Dionne et al.²³ was able to alter the gas composition, but only insulin in the outflow was accessible for analysis. Our system extends these capabilities by enabling the assessment of mitochondrial responses to these perturbations. In addition to oxygen, a number of other gases are important in the study of islet function, including nitric oxide and carbon monoxide, which are thought to be effectors of viability and islet death.^{24,25}

Previous studies of oxygen consumption in islets

Previous studies on oxygen consumption by islets have centered on three approaches: Cartesian diver microrespirometers, continuously stirred chambers, and perfusion systems. The Cartesian diver method involves placing a single islet in a small ampulla containing buffer in contact with a small gas bubble.⁵⁻⁷ The ampule is sealed, and the pressure in the gas bubble is monitored by burette to measure respiration. The continuously stirred chamber, while commonly used for cell studies, has only been used in one study of islet oxygen consumption,³ owing to the potential for the magnetic stirbar to damage the islets. Both the Cartesian diver and the continuously stirred chamber methods take advantage of the fact that continued consumption of oxygen contributes to large changes in oxygen tension that can be easily detected. Disadvantages include an inability to resolve kinetics of consumption or induce sudden changes in composition of the media bathing the islets, the eventual subjection of the islets to hypoxia, and the limitation of experiments to about 2 h. Only one investigator has attempted to make kinetic measurements by perfusion analysis.² Thus, each method has significant technical limitations, which is presumably why only the Cartesian diver method has had repeated use and even this method by only three groups. As has been noted in other studies,³ oxygen consumption measured by perfusion analysis was high. The magnitude of oxygen consumption measured with our flow culture system is also similar to the results obtained with the perfusion system (Table 1). Thus it may be that the continual washing and supply of oxygen to the islet are conducive to maintaining the viability

TABLE 1. OXYGEN CONSUMPTION BY ISOLATED ISLETS BY DIFFERENT METHODS

<i>Method</i>	<i>Species</i>	<i>Oxygen consumption (pmol/min/islet)</i>	<i>Reference</i>
Cartesian diver	Mouse	1.5	5-7
Microelectrode	Mouse	4.2	4
Continuously stirred vessel	Mouse	3.2	3
Perfusion	Rat	8	2
Flow culture	Rat	5-12	Present study

of the islet by reducing the extent of hypoxia to which the islets are subjected. However, it should be noted that our experiments were done with a rich cell culture media. These observations warrant further investigation.

A system for relating mitochondrial function to islet function and death

In this paper we have demonstrated that reduction state of islet cell cytochromes could be detected in our flow culture system (Fig. 7) using techniques first worked out by Chance and Hess.²⁶ With a similar spectrophotometric approach the autofluorescence of NADH could also be measured.²⁷ We envision a future islet assessment system where the electron transport activity will continuously be monitored by simultaneous detection of cytochromes, NADH, and oxygen consumption while fractions are collected for subsequent analysis of insulin and lactate/pyruvate release. This system would be a particularly useful one in studying and assessing mitochondrially mediated islet death.

There is a growing body of evidence that islet death during the process of isolation and transplantation and during the progression of both Type I and II diabetes may be mediated by apoptosis.²⁸⁻³² However, unlike the acute processes of glucose-induced insulin secretion, the progression of apoptosis involving the loss of mitochondrial membrane potential and the translocation of cytochrome c from the mitochondria to the cytosol occurs over the course of hours or days.³³ Thus there is a need for technology that will noninvasively assess kinetically slow changes in mitochondrial function in pancreatic beta cells. In addition, a lack of methods to assess islet quality has been a major impediment to the optimization of human islet isolation techniques for transplantation.³⁴

SUMMARY AND CONCLUSIONS

By adapting an ultrastable oxygen sensor to a flow culture system, acute and chronic changes in oxygen consumption by small numbers of islets can be measured continuously without inducing hypoxic effects. Additional

features of the flow culture system include fine control of composition of media and the ability to simultaneously assess hormone secretion, cytochrome redox state, and other metabolites. Death of islets in culture occurs over days, and a system to continuously monitor the mitochondrial viability can facilitate studies of islet function, optimization of culture conditions, and evaluation of islet quality, all of which may be particularly important to the field islet transplantation.

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