Keywords:
Subcutaneous Ultrafiltration, Diabetes, Glucose Sampling

Monitoring Glucose in Diabetic Mice with the In Vivo Ultrafiltration Diabetes Research Tool Probe

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Purpose

In virtually all diabetes research the most commonly measured variable is glucose. In some cases it is desirable to have a minute by minute profile of the glucose changes. In other cases average values over longer periods of time are more valuable.

Existing Methods

Glucose has traditionally been measured in blood. In small animals there is a limit to the amount of blood which can be removed. There is also the problem of vascular access. Blood can be obtained from mice by cardiac puncture or from the orbital sinus. In rats, blood can be obtained by cardiac puncture or from the tail vein. None of these procedures can be performed easily and without some stress to the animal. The stress of the procedure has the potential of altering the very variable being measured.

Ultrafiltration Method

An alternative to traditional methods of glucose monitoring is the measurement of glucose in ultrafiltrate obtained from a subcutaneously implanted Ultrafiltration probe. It has be shown, in long term studies with dogs, that there is a high correlation of blood and ultrafiltrate glucose in diabetic and normal dogs [1]. The in vivo ultrafiltration (UF) probe can be used to follow the progression of diabetes in naturally diabetic mouse models or following the injection of a diabetogenic drug. The probe can also be used to follow the glucose response to feeding or a glucose challenge.

EXPERIMENTAL

Diabetic Mouse Models

There are several genetic mouse models of diabetes such as the C57/BLKsJ db/db mouse and the NOD mouse. Diabetes can be induced in normal mice with intraperitoneal injection of streptozotocin [2] or alloxan [3].

Ultrafiltrate probes

There are two UF probes which are suitable for implantation in the mouse. The probe with one 2 cm long fiber, UF 1-2 (PN MF-7027) provides a flow rate of 2-4 µL/hour. This is a suitable flow rate to obtain a daily average glucose value. To monitor glucose in the mouse for shorter intervals, the UF 3-2 probe with three ultrafiltrate fibers each 2 cm long (PN MF-7026) provides a flow rate of 8-10 µL/hr [F1]. Note that if the animal is anesthetized or otherwise dehydrated, flow rates are likely to be lower.

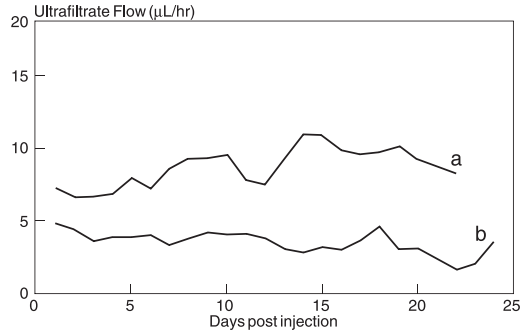

Figure 1. *Flow rate is an important factor in choosing an appropriate UF probe. The UF-3-2 probe provides a flow rate of 8-10 µL/hr (a) and the UF-1-2 provides a flow rate of 2-4 µL/hr (b).*

If long term studies are planned, the probes should be sterilized with ethylene oxide to prevent degradation of the glucose in the ultrafiltrate sample by bacterial contamination. We suggest that the probes be implanted within several days of ethylene oxide sterilization since it can cause drying of the glycerol in the membrane pores, which will lead to a reduction in probe permeability.

Sample Collection

The negative pressure for sample collection can be generated in two ways. A Vacuum Needle Holder (MD-1322) can be attached to the UF probe tubing. The needle is inserted into a Vacutainer® (PN MF-7024). The Vacutainer® serves as the vacuum source and sample vial. Alternatively the probe tubing can be attached to a peristaltic pump , in which case the samples can be collected automatically in a refrigerated fraction collector (PN MD-1201). For long term studies, where the goal is to determine the progression of diabetes and only one or two samples a day are required, the Vacuum Needle Holder and Vacutainer® system is preferable. The Vacutainers® are sterile and the Vacuum Needle Holder can be ethylene-oxide sterilized. For short term studies which require frequent sampling, the pump and fraction collector may be preferable.

Ultrafiltrate Probe Implantation

The subcutaneous UF probes are implanted according to the procedure described elsewhere [4]. Mice are anesthetized with an intraperitoneal injection of 0.01 mL/kg of KX (10 mL Ketamine (100 mg/mL) + 1 mL Xylazine (100 mg/mL). A 1/3 cc insulin syringe (Becton-Dickenson) is useful for this injection.

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The fur is clipped from the implantation site on the back at the base of the neck. The probe is placed in the introducer cannula (PN MR-5313). A small incision is made at the insertion site. The cannula is inserted under the skin and withdrawn, leaving the fiber portion of the probe under the skin. The probe is sutured in place. In order to prevent the mouse from chewing or scratching a hole in the probe tubing, a three-inch piece of lightweight spring or hard plastic tubing can be placed over the probe tubing. A collar (PNMD-1365) is placed around the neck of the mouse.

For Vacutainer® collection, insert the probe tubing into the Vacuum Needle Holder assembly and insert the needle into the Vacutainer®. The mouse is placed in an awake animal sampling caging system such as the MD-1570. The collar of the mouse is tethered to a lightweight lever system and the Vacutainer® is secured to the tether [2]. An acrylic cover is placed over the container to prevent the escape of the mouse.

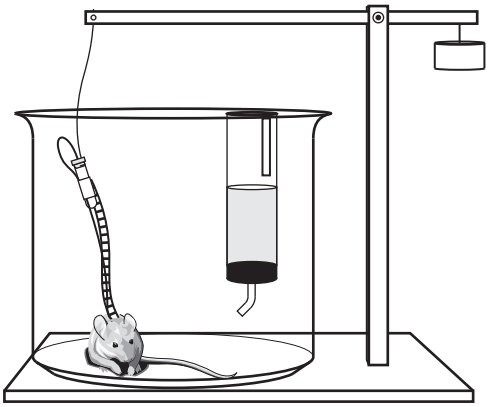

Figure 2. *Awake animal system for mouse with a UF probe.*

For automatic fraction collection, the mouse is placed in a animal sampling system (PN MD-1570 or MD-1409) and the probe tubing is attached to the swivel with a tubing connector (PN MF-5163)or out through the Raturn sensor assembly.

Glucose Analysis

Glucose can be analyzed by biosensor or flow injection analysis using a chromatograph with an immobilized enzyme reactor column (Contact BASi for details). For the chromatographic approach, the column should be maintained at 35 °C by an LC-22A temperature controller with an LC-23A column heater. The mobile phase is 20 mM NaH_2PO_4 pH 5.5 with 5 mL/L of BASi ProClinTM 150 reagent (CF-2150). The flow rate is 1 mL/min. For electrochemical detection a platinum electrode is maintained at + 500 mV vs. Ag/AgCl. The electrode is coated with Nafion® to minimize interferences [5-6]. UF samples are diluted 50 to 100 times with mobile phase, depending on the concentration of glucose in the samples. For analysis by biosensor contact BASi for details.

Results and Discussion

The technique of monitoring glucose levels utilizing the UF probe as a diabetes research tool allows the documentation of the degree of exposure to hyperglycemia which each animal has sustained. This is valuable information in correlating the effect of hyperglycemia on long-term complications or evaluating drugs or treatments. F3 shows the glucose history of three mice treated with the same dose of streptozotocin. The degree of diabetes induced in the three mice was quite different.

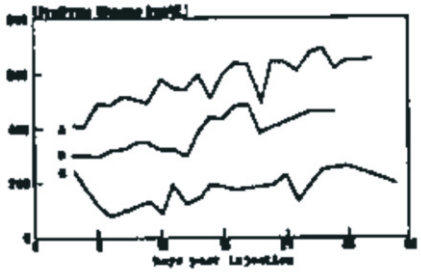

Figure 3. *Three mice develop different levels of hyperglycemia after injection of streptozotocin.*

F4 shows that the variation in glucose levels in a single mouse over a 12 hour period can be two hundred mg/dL. Because of these fluctuations, a few isolated blood glucose values may not be a valid indication of the degree and duration of exposure to hyperglycemia.

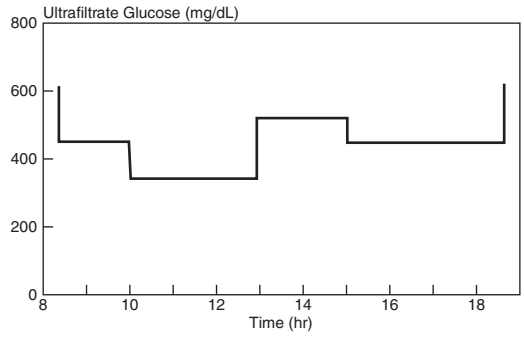

Figure 4. *Glucose levels can fluctuate over 200 mg/dL in the course of a day.*

References

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** The UF probe and Capillary Filtrate Collector are protected by US Patent Nos. 4,777,953, 4,854,322, and 5,002,054.*