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Tech Notes

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Glucose Tolerance Test Using Ultrafiltration Sampling and the Rodent Workstation

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The glucose tolerance test is the gold standard in diagnosing diabetes in humans and animals. It is also used extensively in diabetes research to evaluate new drugs, test new devices and to study basic physiology and pathology of diabetes. It is also useful to evaluate the effect of drugs on glucose tolerance. Some drugs, which are developed for purposes other that treatment of diabetes, also have an effect on glucose tolerance and may be potentially dangerous if used by diabetics if the effects on glucose are not known. Therefore, the effect of all new drugs on glucose tolerance should be investigated regardless of intended use of the drug. Botanical products, which have also become very popular, may have an effect on glucose concentrations and the glucose tolerance test is a useful tool for botanical investigations.

Much of the diabetes and drug research is done in rodents, which poses special sampling problems. Handling rodents to obtain samples can cause stress and this stress can change blood glucose values obscuring the effects of the test variables. The traditional way of evaluating glucose during a glucose tolerance test is with blood sampling. Because of the small size of rodents there is a limit to the amount of blood that can be withdrawn and therefore the frequency of sampling. If multiple glucose tolerance tests are required it may be necessary to wait 2 weeks between tests to allow the rodents to replace the depleted blood volume.

Ultrafiltration sampling provides a useful alternative to blood sampling. The ultrafiltration probe is placed subcutaneously and interstitial fluid is removed with a negative pressure, which is applied with a pump or Vacutainer. The glucose concentration in interstitial fluid is the same as in blood. The advantage of using ultrafiltrate probes to obtain samples in glucose tolerance tests is that there is no blood loss. Therefore, there is no restriction on the number of samples or the frequency with which tests can be repeated. This technique is also very useful if studies are conducted over longer periods of time. If blood sampling is used and catheters fail, it may not be possible to get all of the data. Because ultrafiltration probes are subcutaneous, they can be easily replaced if they should fail.

Glucose tolerance tests are best preformed in a Rodent workstation. The Rodent Workstation uses Raturn rodent housing system, which automatically compensates for turning movements to prevent twisting of lines and catheters. Samples can be taken with out handling the rat. A refrigerated fraction collector collects and stores samples at 4°C.

In this example lean and diabetic Zucker rats were given baseline glucose tolerance tests. They were dosed with Amaryl, a drug used to treat type 2 diabetes, and the glucose tolerance tests were repeated.

Materials and Methods

Rats: In this case male Zucker Lean (+/+ or +/fa) and diabetic (fa/fa) rats from Charles Rivers were used. These rats are a model for type 2 diabetes. They begin to become obese and diabetic at 7 weeks of age.

Probe implantation: The rats were anesthetized with isofluorane. UF-3-12 ultrafiltrate probe (PN MF-7023) were unplanted subcutaneously (See BASi Tech Notes 1003 for implantation instructions.) A collar was placed on the rats and the ultrafiltration probes were attached to a vacutainer. The rats were put into the Rodent Workstation. The balanced arm was attached to the collar and the vacutainer tubes were placed on the shelf above the Raturn and secured with tape. The animals were allowed to recover for 2 days.

Glucose Tolerance Tests: Rats were fasted overnight before glucose tolerance tests. Before beginning the glucose tolerance tests the ultrafiltrate probe was switched from the Vacutainer to a peristaltic pump. The outflow tubing was attached to the BASi Culex Refrigerated Fraction Collector (PN MD-1201) and the sampling time was set to 15-minute intervals. Glucose was administered as a 50% solution at a dose of 1ml/1kg of body weight by gavage. After administration of glucose the rat was returned to its cage and sampling proceeded automatically.

A baseline glucose tolerance test was done on each rat with no drug. The diabetic rat was then given Amaryl for 2 days before the next glucose tolerance test at a dose of 0.11 mg/kg. On the day of the second glucose tolerance test, the rats were give a 0.11g/kg dose of Amaryl 2 hours before the start of the glucose tolerance test.

Results and Discussion

Figure 1 show the glucose tolerance tests in a lean normal rat and figure 2 shows the glucose tolerance tests in a fatty diabetic rat. In these tests the peak glucose concentrations are seen at 45 minutes. In glucose tolerance tests with blood sampling the peak usually appears at 15 minutes. The difference in time is due to the time it takes the sample to traverse the dead volume of the tubing between the subcutaneous tissue and the fraction collector. It is possible to correct the times for the dead volume but it is not necessary if comparing glucose tolerance tests taken under the same conditions.

In this case the lean rat has a fasting glucose around 5 mM. After glucose dosing it rises to 7.8 mM and returns to slightly below baseline 30 minutes later. This is typical normal glucose tolerance curve. When the lean rat is given Amaryl before dosing with glucose there is no rise in glucose concentration, indicating that this drug is having an effect even in the normal animal. In the diabetic rat, the fasting glucose is about 15mM. After glucose dose it rises to 22mM.

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The glucose in this case dose not return to normal quickly as it did in the lean rat, but remains elevated for an hour and a half. It then declines but does not return to starting concentrations. This is a typical pattern for a diabetic rat or human. Treatment with Amaryl for two days lowered the fasting glucose to about 10 mM. The glucose load raised the glucose concentration to 17.5 mM. The glucose in this test did not remain as high for as long as in the untreated test. This indicates that Amaryl is useful in treating diabetes but for this case the dose was not adequate to normalize glucose tolerance.

The ultrafiltration sampling method and the Raturn Workstation are useful tools in diabetes research, whether it is for drug development, nutraceutical evaluation, metabolic studies or device development. Use of ultrafiltrate instead of blood allows for more intensive sampling and longer term studies.

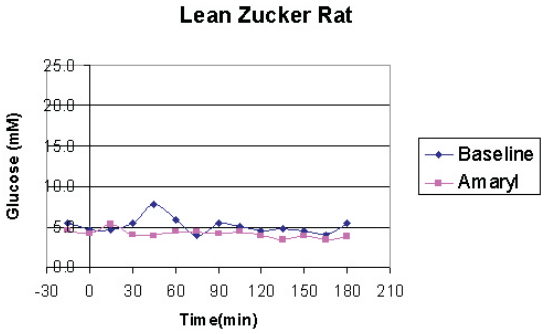

Figure 1. *Baseline glucose tolerance tests with no drug and after dosing with Amaryl in a Zucker Lean rat.*

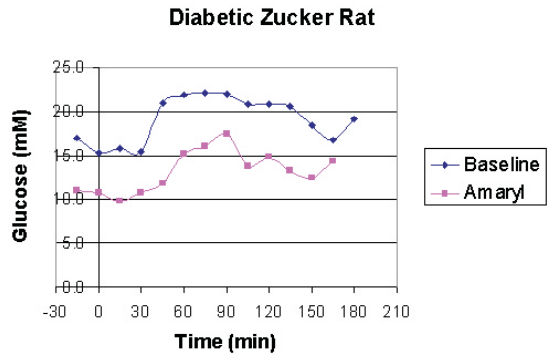

Figure 2. *Baseline glucose tolerance tests with no drug and after dosing with Amaryl in a Zucker Diabetic rat.*

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