Rodent Models of Diabetes

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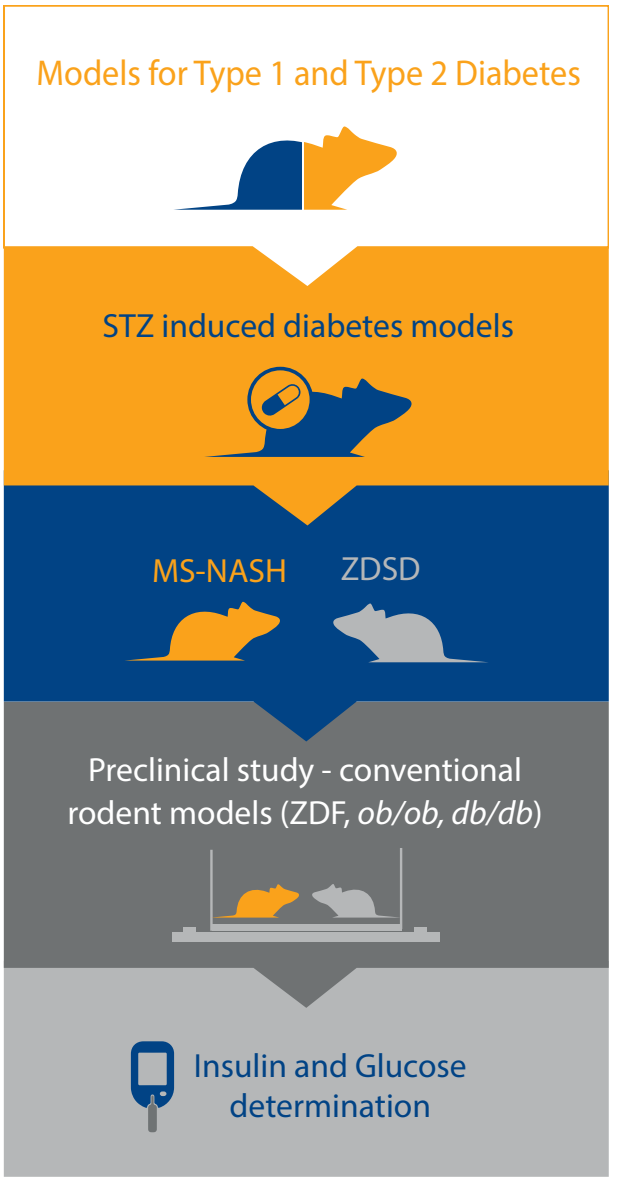
Enhance your preclinical diabetes research with CrownBio's conventional and highly translatable rodent models

Discover how our models, combined with key glucose and insulin determinations, can drive forward your anti-diabetic agent development programs.

Rodent models are a key first stage in *in vivo* preclinical translational studies, providing important information on disease mechanisms, efficacy, and safety to make early stage go/no-go decisions on which compounds should progress to further study in larger mammals before clinical trial. Appropriate preclinical rodent models and technologies are needed for diabetes research to evaluate novel agents treating the progressive disease and related complications.

CrownBio provides a comprehensive rodent diabetes research platform, including:

- Conventional rat and mouse models of Type 1 and 2 diabetes such as ZDF and ZSF rats and *ob*/*ob* and *db*/*db* mice, including induced disease.
- Highly translational models of Type 2 diabetes including the MS-NASH (formerly called FATZO) mouse, and ZDSD rat – the most translatable rodent models for diabetes research.
- Key diabetes endpoint monitoring and analyses, including glucose and insulin determinations.



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Rodent Models of Diabetes Key Facts

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Our comprehensive rodent diabetes research platform is used to progress anti-diabetic agent development:

- With monitoring of two key endpoints in diabetes research
  - Glucose determination via single point or continuous telemetry monitoring, combined with glucose and insulin tolerance tests.
  - Insulin determination via ELISA based assays.
- In highly translatable polygenic models of Type 2 diabetes – the MS-NASH mouse and ZDSD rat which closely mimic the human condition without leptin/leptin receptor mutations.
- As well as conventional and induced diabetes models for consistency with historical research
  - Spontaneously occurring Type 1 diabetes models NOD mice, BB rat, NRG-Akita.
  - STZ induced diabetes rat and mouse models.
  - Type 2 diabetes rat models (ZDF and ZSF-1) and mouse models (*db/db* and *ob/ob*).

CrownBio Diabetes Technologies and Resources

The two key endpoint analyses CrownBio provides for diabetes research are glucose and insulin determination. Glucose determinations are performed via single point or continuous telemetry monitoring, as well as through glucose and insulin tolerance tests. Insulin determination is performed via ELISA based assays.

We also provide glucose clamp tests, which are the gold standard for quantifying insulin resistance. These techniques can be utilized in any commercially available rat or mouse model, allowing consistency with historical data where required. We can perform studies in streptozotocin (STZ) induced diabetic rat and mouse models. A single injection can be used to develop a suitable level of diabetes in rats; however, in mice two to three STZ injections are required to create a stable model.

Studies can also be run using models of Type 1 diabetes with spontaneously occurring disease (NOD mice, the BB rat, and the NRG-Akita mouse with immune deficiency, run in a sterile environment). For Type 2 diabetes, commercially available models include ZDF and ZSF-1 rats and *db*/*db* and *ob*/*ob* mice.

We also provide highly translatable models of Type 2 diabetes including the MS-NASH mouse and ZDSD rat. These are polygenic models of dysmetabolism, spontaneously developing metabolic syndrome, obesity, and diabetes, which more closely mimic the human condition than models with leptin/leptin receptor mutations.

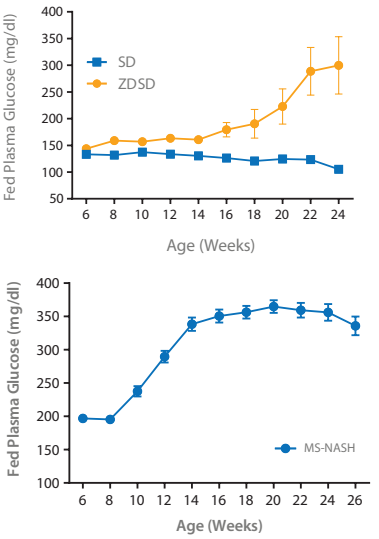
Male MS-NASH mice become hyperglycemic at 14 weeks of age, and also develop insulin resistance at an early age. The ZDSD rat progresses through Type 2 diabetes similarly to humans, with a *bona fide* pre-diabetic state occurring (pre-diabetes 8-16 weeks of age, overt diabetes >16 weeks of age, diabetic complications 24+ weeks of age). Both models respond to anti-diabetic treatments and can be used in their evaluation.

The ZDSD rat can also be used to research diabetic complications including but not limited to nephropathy, neuropathy, and fatty liver.

Glucose Determination

Single time point glucose determinations are performed using a glucometer and a glucose strip (Nova® Biomedical StatStrip® Xpress), which has been validated in rodents to an upper limit of 900mg/dl. This is high enough to measure glucose levels even in severely diabetic Type 1 animals. **Figure 1** shows fed glucose determined using this method in the highly translatable Type 2 diabetic ZDSD rat compared with the conventional SD model, as well as in the Type 2 diabetic MS-NASH mouse. As a standard, single point measurements are taken around 6:00 to 8:00 am.

Figure 1: Fed Glucose Determined in ZDSD and SD Rats, and in MS-NASH Mice, using Single Point Glucometer Glucose Strip Determination



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CrownBio also provides glucose determination via continuous measurement using DSI telemetry technology. The technique is available for both rat and mouse models. The telemetry device probe is implanted into the aorta of the animal, and is coated with glucose oxidase to monitor glucose concentration. The device can be used for approximately four week studies (limited by the probe lifetime which is based on the amount of glucose oxidase which can be loaded). Body temperature can also be measured using the telemetry device if required.

Continuous glucose monitoring is beneficial when events need to be collected over time, especially when monitoring diurnal glucose excursion to understand which phase of glucose homeostasis is being effected by a drug under evaluation. Continuous monitoring also shows biochemically and physiologically what is happening to glucose in the study animal over time, and the types of systemic glucose lows the model is exposed to throughout the day. **Figure 2** shows typical continuous glucose monitoring recordings in the ZDSD rat and response to the addition of metformin. An expanded version of this data recording diurnal glucose excursion over three days is displayed in **Figure 3**, showing the valuable data that continuous glucose monitoring readings over multiple 24 hour periods provides. Glucose values are shown to greatly fluctuate over a given 24 hour period, with early evening hyperglycemia recorded. Drug effect throughout the day can be studied, and points of hyperglycemia recorded that may be missed by early morning single time point measurements.

Figure 2: Continuous Glucose Monitoring in the ZDSD Rat Showing Response to Metformin

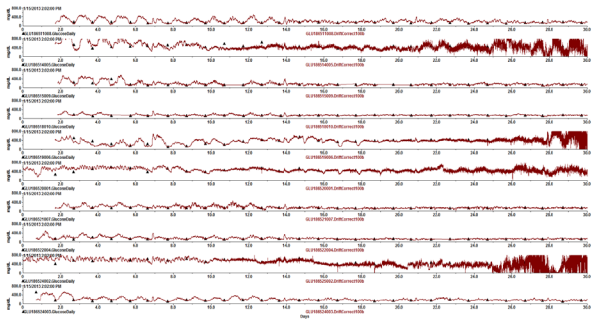
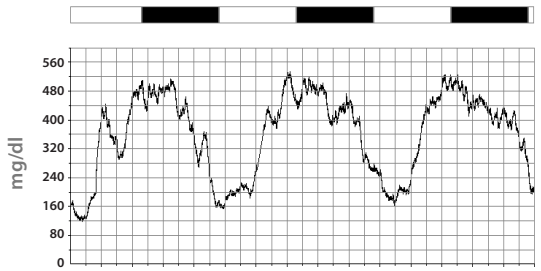


Figure 3: Continuous Monitoring of Diurnal Glucose Excursion in the ZDSD Rat over 3 Days

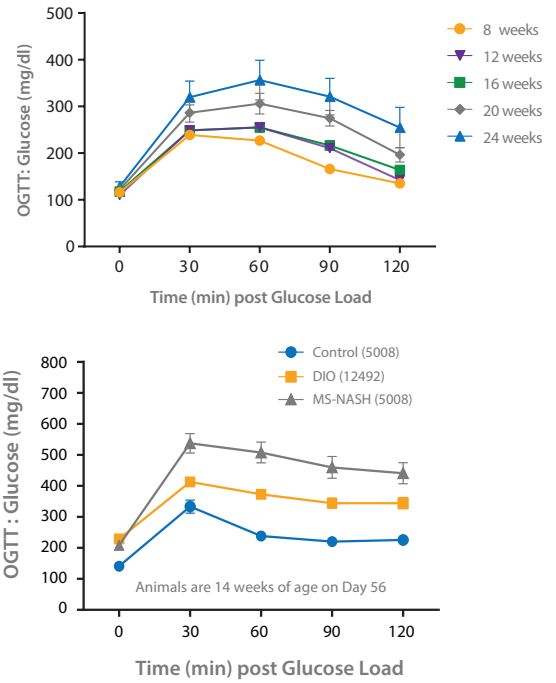
White bar: daylight hours. Black day: nighttime hours.



Glucose Tolerance Tests

Glucose tolerance tests (GTT) are usually performed by oral administration of 2.0g/kg glucose to study animals; we also provide i.v. or i.p. GTT as required. As a standard, five to six time points are studied to construct a time course of the glucose response, using single point glucometer measurements. Example data in ZDSD rats, and MS-NASH, diet induced obesity (DIO), and control mice are shown in **Figure 4**. Control animals with regular insulin function quickly normalize their glucose levels following glucose challenge, whereas higher levels of glucose are maintained in validated diabetic animals (such as the MS-NASH mouse) with impaired insulin function. DIO models which are insulin resistant but not hyperglycemic show only a moderate increase in glucose levels in comparison with control. In the ZDSD rat following glucose challenge, glucose levels remain higher in older animals which show a more highly diabetic phenotype and greater insulin resistance, which is highly typical of diabetic models.

Figure 4: Oral Glucose Tolerance Tests in the ZDSD Rat, and MS-NASH and DIO Mice with Single Point Measuring



For a more thorough review of the GTT response, CrownBio can produce continuous glucose curves, constructed using the invasively implanted DSI telemetry glucose sensor described above. **Figure 5** shows OGTT in the ZDSD rat treated with vehicle or metformin with continuous glucose monitoring over 3 hours, with metformin functioning to allow the ZDSD rat to normalize glucose levels.

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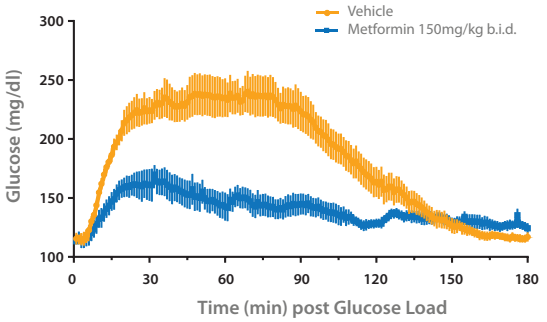
Figure 5: Metformin Reduces Glucose Levels in ZDSD Rats OGTT Recorded by Continuous Glucose Monitoring over 3 Hours
16 week old ZDSD rats, n=6, mean ± SEM. Data represent a data point every minute for 3 hours post-glucose administration.



Insulin Tolerance Tests

Insulin tolerance tests are conducted by following glucose response to the administration of insulin (i.p. or s.c.) over five to six single time points (example data shown in **Figure 6** for SD rats) or by continuous glucose monitoring (example data shown in **Figure 7**) as previously detailed. **Figure 7** compares the response to challenge with a fast acting (Humulin® R) and slow acting (Humulin NPH) insulin. The glucose nadir occurs at approximately the same time point for both insulin products, with the fast acting insulin providing a more rapid return to normal glucose levels. Continuous monitoring allows full data collection, including early data that may be missed if single time point monitoring protocols begin only 1 hour post-insulin dosing.

Figure 6: Insulin Tolerance Test in the SD Rat with Glucometer Single Point Measuring
Insulin administered s.c., n=4.

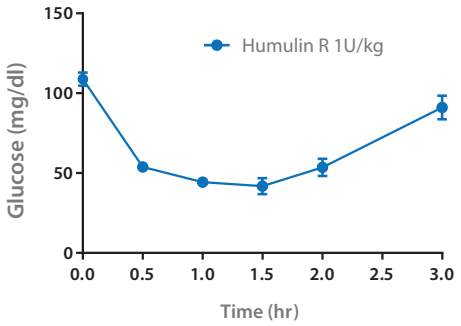
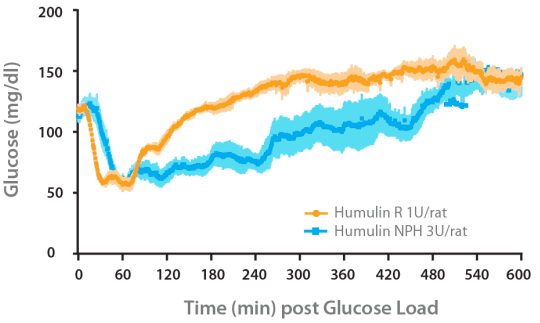


Figure 7: Insulin Tolerance Test in Rats with Continuous Glucose Monitoring n=6, mean ± SEM.



Insulin Determination

A further key endpoint provided by CrownBio is the determination of basal insulin levels. Both plasma and serum insulin levels can be measured in a variety of rodent models using ELISA based chemiluminescence 96 well plate technology or via MesoScale Discovery electrochemiluminescence detection.

Summary

CrownBio provides monitoring of the two key endpoints in diabetes research: glucose and insulin determination. Glucose measurements, as well as glucose and insulin tolerance tests, can be performed as single time point measurements using a glucometer or as continuous telemetry monitoring. Continuous monitoring is beneficial when events need to be collected over time, and also fully elucidates what is happening to glucose in the study animal over an extended period and following drug treatment. Insulin determination is performed via ELISA based assays.

These techniques can be utilized in any commercially available rat or mouse model (e.g. ZDF and ZSF-1 rats, *db/db*, *ob/ob,* and NOD mice) as well as CrownBio's proprietary models, the ZDSD rat and the MS-NASH mouse. The polygenic ZDSD rat and MS-NASH mouse models are highly translational because they more closely mimic the human disease than commonly used conventional monogenic models with leptin/leptin receptor mutations.