Diabetes models

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Diabetes model concept
The diabetes

Type 1
≈2 à 4% of human population
- Inability to produce insulin (destruction of β cells).
- Autoimmunity → genetic / virus / food.
- Dietary and insulin therapy / education.

Type 2
≈5% / 50% of human population
- Insulin resistance.
- Cell dysfunction β.
- Genetic → lifestyle.
  - FOOD ???
  - Dietary + physical activity +
  - education / R → insulin therapy

Importance of prevention

Type I Diabetes
(In human disease)

- Juvenile diabetes
- Insulin-dependent diabetes mellitus
- Autoimmune disorder
- The body destroys the cells that make the insulin
- Thus, the body can no longer produce insulin
Type II Diabetes

- Non-insulin dependent diabetes mellitus
- Body makes insulin but most cells do not use insulin properly
- **Insulin resistance**
- Slowly, the pancreas stops making insulin.

Complications of Diabetes

- Vascular damage
- Heart disease
- Kidney damage
- Diabetic neuropathy / nerve damage
- Skin and mouth conditions (bacterial and fungal infections)
- Foot damage
- Pregnancy complications
Metabolic models (diabetes/obesity)

- Models:
  - BKS(D)\(\text{Lepr}^{\text{db}}\)/JOrIRj: BKS Diabetic mouse
  - B6.V\(\text{Lep}^{\text{ob}}\)/JRj: B6 Obese mouse
Diabetic Mutant

Diabetic Model:
BKS(D)-\textit{Lepr}^{db}/JOrIRj

Origin: \textit{db} mutation occurred in a colony of inbred C57BL/Ks (HUMMEL, 1966).

The mutation is located on chromosome 4. Effect: Lack of leptin receptor

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BKS Diabetic mouse strain

- **Features:**
  - Expresses obesity around 4 to 5 weeks of age
  - Elevated plasma insulin from 10-14 days
  - Hyperglycemia from 4 to 8 weeks of age
  - Polyphagia, proteinuria, glucosuria, polyuria, polydipsia
  - Hyperinsulinemia despite a severe impairment of \( \beta \) cells of the islets of Langerhans
  - Deficiency of the leptin receptor
BKS Diabetic mouse strain

- Using the mouse obese for research:
  - Type 2 diabetes
  - Peripheral neuropathy
  - Diseases of the myocardium
  - Immunodeficiency
  - Immunology
  - Metabolism
  - Obesity

The db/db mice is highly hyperglycemic
Plasma glucose and HbA1C

![Graph showing glucose and HbA1C levels over time for db/db and db/+ mice]
The Obese Mutant

Obesity Model:

B6.V-Lep^{ob}/JRj

Cause: An autosomal recessive mutation in the leptin gene on chromosome 6, which appeared on the strain V from JACKSON LABORATORY in 1949.

Effect: Lack of leptin

Obese mutant strain

• Features:
  – Obesity phenotype is expressed from 4 to 5 weeks of age.
  – Hyperphagia, Hyperinsulinemia, Impaired, hyperlipemia
  – Lack of regulation of body temperature
  – Hyperplasia of adipocytes
Obese mutant strain

- Using the mouse obese for research:
  - Obesity
  - Diabetes
  - Metabolism
  - Gerontology
  - Nutrition

Obese mutant strain: Growth curve
The ob/ob mouse is hyperglycemic
Plasma glucose and HbA1C

Moderate hyperglycemia (15-20 mmol/l) and a slight glucose intolerance that appears with aging
Very strong hyperinsulinemia and a preserved insulin response to glucose
Insulin resistance that worsens with aging as showed by insulin and glucose profiles evolution
Deteriorated lipid profile
Inflammation

The db/db mouse model
Strong hyperglycemia (20-30 mmol/l) and glucose intolerance that appears early
Hyperinsulinemia and no insulin response to glucose showing a strong defect in beta cell function
Insulin resistance (hyperglycemia/hyperinsulinemia)
Deteriorated lipid profile
Inflammation

Final summary
Mouse model characteristics
Spontaneous diabetic animals:

Advantages

- Development of type 2 diabetes is spontaneous origin involving genetic factors and the animals develop characteristic features resembling human type 2 diabetes
- Mostly of inbred animal models in which the genetic background is homogeneous and environmental factors can be controlled, allowing genetic dissection of this multifactorial disease easy
- Variability of results perhaps minimum and require small sample size

Disadvantages

- Highly inbred, homogenous and mostly monogenic inheritance and development of diabetes is highly genetically determined unlike heterogeneity seen in humans
- Limited availability and expensive for the diabetes study
- Mortality due to ketosis problem is high in cases of animals with brittle pancreas (db/db, ZDF rat P. obesus, etc.) and require insulin treatment in later stage for survival
- Require sophisticated maintenance
Diet induced obesity models (DIO)

- Experimental diets used:
  - They are characterized by a high energy intake of various origins (Lipids, cholesterol, carbohydrates, proteins).
  - The power will quickly induce obesity and cause the development of type 2 diabetes (NIDDM).
  - The period between the beginning of the plan and the observation of clinical symptoms largely depends on the strain of mice used and fat intake.
The DIO test

DIO: Diet Induced Obesity

• Not just environmentally caused, genetics are involved in the generation of a DIO mouse
• Pay Attention!: (Only some strains are sensitive to DIO)
• Pay Attention!: (C57BL/6 is the strain of choice for generating DIO mice)

The C57BL/6

Mutation in nnt gene

• Characteristics
  – Decreased Energy Expenditure
  – Hypercholesterolemia
  – Hyperglycemia
  – Hyperinsulinaemia
  – Hyperlipidemia
  – Hyperphagia
  – Insulin resistance
SPRAGUE DAWLEY

- Characteristics:
  - Hyperleptinemia
  - Hyperphagia
  - Hypertension
  - Leptin resistance
  - Mild insulin resistance

Rj Han:SD

WISTAR

- Characteristics:
  - Hyperleptinemia
  - Hyperphagia
  - Hypertension
  - Leptin resistance
  - Mild insulin resistance

- SD is more reactive than Wistar

Rj Han:WI
Diet/Nutrition induced diabetic animals:

- **Advantages:**
  - Develop diabetes associated with obesity as a result of overnutrition as in diabetes-obesity syndrome like in human population
  - Toxicity of chemicals on other vital organs can be avoided
Diet/Nutrition induced diabetic animals:

- Disadvantages:
  - Mostly require long period of dietary treatment
  - No frank hyperglycaemia develops upon simple dietary treatment in genetically normal animals and hence become not suitable for screening antidiabetic agents on circulating glucose parameter

Chemical induced diabetes
The Streptozocin (STZ)

- Antibiotic produced by mitotic *Streptomyces achromogenes*.
- Alkylating agent acting on the DNA methylation.
- Interferes with glucose transport, glucokinase and induces multiple breaks of the DNA strand.
- Glucose transporter β cells of the islets of Langerhans gathers enough to cause necrosis active cell specific.

STZ rat: examples

- Sprague Dawley/Wistar from 150 to 200 g
- Standard food
- 1 dose of 65 mg/kg

⇒ Type 1 Diabetes (Wu and Huan, 2008)
Type II diabetes model

• Model of type II diabetes by streptozotocin injection in neonate rat = n0-STZ

• Tool for long-term study on the consequences of a gradual reduction of the pancreatic beta cell mass

• Similarity between the insulin secretion defect and its action in this model and the one described in the human type II diabetes

Type II diabetes model – n0-STZ: Study - Protocol

• Injection of 100 mg/kg STZ in saphenous vein at J0 in neonate male Wistar RjHan/WI

• Control n = 8, n0-STZ n = 16
  – Measurements:
    • Glycemia at D4
    • Glucose tolerance test at D56
    • Weight curve
Induce Diabetes type I or II

Streptozotocin-Induced Diabetic Models in Mice and Rats

Brian L. Furman

Streptozotocin (STZ) is an antibiotic that produces pancreatic islet β-cell destruction and is widely used experimentally to produce a model of type 1 diabetes mellitus (T1DM). Detailed in this unit are protocols for producing STZ-induced insulin deficiency and hyperglycemia in mice and rats. Also described are protocols for creating animal models for type 2 diabetes using STZ. These animals are employed for assessing the pathological consequences of diabetes and for screening potential therapies for the treatment of this condition.

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Strain STZ susceptibility
Strain use for STZ protocol

- **Rats:**
  - Sprague Dawley
  - Wistar

- **Mouse:**
  - C57BL/6
  - ...

Chemical induced diabetic animals:

**Advantages**

- Selective loss of pancreatic beta cells (alloxan/STZ) leaving other pancreatic alpha and delta cells intact
- Residual insulin secretion makes the animals live long without insulin treatment
- Ketosis and resulting mortality is relatively less
- Comparatively cheaper, easier to develop and maintain
Chemical induced diabetic animals: disadvantages

- Hyperglycaemia develops primarily by direct cytotoxic action on the beta cells and insulin deficiency rather than consequence of insulin resistance.
- Diabetes induced by chemicals is mostly less stable and at times reversible because of the spontaneous regeneration of beta cells. Hence, care must be taken to assess the pancreatic beta cell function during long-term experiments.
Lund METS rat

- **METS** for METabolic Syndrome
- Congenic rat
- Genetic background: Bio Breeding Diabetes Resistant (BBDR)
- BioBreeding is not commercialised
- Created by Lund University

• Male animals express two phenotypes
  - Severe diabetes (D)
  - Moderate prediabetes (M)
Some main physiologic parameters

BBDR.cg-lepr.cp model

Is a good study model for

- Obesity
- Hyperglycaemia
- Cardiomyopathy
- Nephropathy
- Hyperlipemia
- Hepatosteatosis and hepatomegaly
- Vascular hypertension
A good model for human diabetes and associated diseases

Transgenic/knock out diabetic animals:

- **Advantages**
  - Effect of single gene or mutation on diabetes can be investigated *in vivo*
  - Dissection of complex genetics of type 2 diabetes become easier

- **Disadvantages**
  - Highly sophisticated and costly procedure for the production and maintenance
  - Expensive for regular screening experiments
Type I Diabetes spontaneous model

• The non-obese diabetic (NOD) mouse
• bio breeding (BB) rat
• LETL (Long Evans Tokushima lean) rat

are the two most commonly used animals that spontaneously develop diseases with similarities to human Type 1 diabetes.

Type II Diabetes mutant model

• Zucker (fa/fa) rat—monogenic model of obesity (leptin resistant)
• ZDF rat
• Goto Kakizaki rat
• KK mouse
• NSY mouse
• OLETF rat
• Israeli sand rat
• CBA/Ca mouse
• Diabetic Torri rat
• New Zealand obese mouse
Thank you for your attention