Diabetes Mellitus & the Cardiometabolic Syndrome in Swine

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Introduction
Diabetes mellitus is occurring in epidemic proportions worldwide and it is estimated by the World Health Organization (WHO)1 and the American Diabetes Association (ADA)2 that 300 million persons will have the disease by 2025. Currently WHO and ADA classify the main types of diabetes mellitus as Type 1 (formerly juvenile-onset or insulin-dependent) or Type 2 (formerly adult-onset or non-insulin-dependent).

Type 1 diabetes mellitus is an autoimmune disease which is mediated by T cell destruction of insulin secreting beta cells in the islets of Langerhans of the pancreas (Figures 1-2). The autoimmune insult is associated with genetic predisposition, infections and/or toxins. Type 2 diabetes mellitus is responsible for approximately 90% of the human cases. It is associated with insulin resistance or reduced insulin sensitivity combined with reduced secretion of the hormone. It increases in onset with age and is associated with central visceral obesity and inheritance. There are other forms of the disease associated with pregnancy (gestational diabetes), drugs, toxins or diseases that affect the pancreas. In all of its forms it is a chronic disorder associated with hyperglycemia and abnormal metabolism of carbohydrate, fat and protein.1-4

Diabetes mellitus is associated with damage of blood vessels leading to a variety of complications. Included in these are accelerated atherosclerosis, coronary artery disease, stroke and peripheral vascular disease. Diabetes also damages small blood vessels which can lead to retinopathy, neuropathy and nephropathy due to microangiopathy.1-4

Figure 1. Histologic section of the pancreas (H & E, X 100)

The cardiometabolic syndrome is closely related and is associated with an increased risk of cardiovascular disease. There is a cluster of risk factors including insulin resistance, decreased glucose tolerance, dyslipidemia, central obesity, microalbuminuria, hypercoagability and hypertension. There remains some controversy concerning the precise set of symptoms which
constitute the syndrome but generally having a combination or three or more of these characteristics constitutes a diagnosis of the syndrome. The syndrome is associated with stroke, congestive heart failure, chronic kidney disease, endothelial dysfunction and chronic kidney disease.\(^1\)\(^-\)\(^5\)

Diagnostic Criteria

Human: Type 1 diabetes mellitus is characterized by a deficiency of insulin as compared to increased insulin resistance or reduced insulin sensitivity and/or reduced insulin secretion in the Type 2 form. There is now an overlap in ages but Type 1 is generally in the young versus a more mature population for Type 2. In particular Type 2 is increasingly found in the young. In all cases it is associated with elevated levels of fasting plasma glucose levels >126 mg/dl (>7 mmol/l) whereas the normal is <110 mg/dl. Testing of plasma glucose is preferred over use of the glucose tolerance test but elevations >200 mg/dl (11.1 mmol/l) following a 75 g oral glucose feeding is also diagnostic. Clinical symptoms of the recent onset type include polydipsia and polyuria accompanied by weight loss. However, the chronic onset may be more insidious with complications of diabetes being the predominant sign. Glucose is also passed in the urine. Glycosylated hemoglobin (Hb A 1c) reflects average blood glucose readings associated with glucose bound to hemoglobin over the past 90 days and is mainly used as criteria for tracking effective treatment with insulin (abnormal >6%).\(^1\)\(^,\)\(^2\)

Swine: Spontaneous cases of diabetes mellitus are rare in swine and genetic models are not commercially available. Diabetes may be
induced either surgically or chemically. Dyslipidemia may also be induced by dietary manipulations as a complication of the disease. There is some variation in plasma glucose levels between various breeds of minipigs and slight gender related variations with the females being slightly higher than males. Similar diagnostic criteria as used for humans may be used for swine. Most authors give a range of 70-90 mg/dl as a mean value for fasting blood glucose. Some normal animals may range as high as 150 mg/dl but most will fall <130 mg/dl. As a generality, fasting swine have insulin levels of 5-10 μU/ml which will increase 2-3 times that amount following a meal. Normal cholesterol values for minipigs on atherogenic diets are typically <100 mg/dl with triglycerides <60 mg/dl. HbA1c is not as reliable in swine as it is in humans. In the research setting every animal should be used as its own control and normal blood levels for the individual should be determined prior to induction of diabetes.4-12

Induction of Diabetes
Type 1 diabetes mellitus can be produced either surgically or chemically. It is less technically challenging to induce the syndrome with alloxan or streptozotocin instead of performing a pancreatectomy. There are differences between breeds and between ages within the same breed, however, most authors recommend alloxan 100-200 mg/kg iv or streptozotocin 140-150 mg/kg iv. Either injection should be given as a continuous infusion in saline over a 5-10 minute time period in fasted animals. Complications may occur either acutely or chronically following the injection. Acute insulin release may result in fatal hypoglycemia. There can also be toxic reactions resulting in hepatic or renal failure. Hyperglycemia will be apparent within a few days if the ablation is successful. Beta cell mass relative to body mass is approximated 2 times more than in humans. If regeneration of Beta cells occurs it will usually be apparent within 1-2 weeks and the injections will have to be repeated. Other schemes of delivery of streptozotocin have been described including dividing the dosage into 2-3 portions and repeating the injections over several days to weeks.4-12

Surgical induction may be accomplished by performing a total pancreatectomy.4-12 However, this will produce exocrine dysfunction in addition to the endocrine dysfunction. Pancreatectomy is a major procedure which requires advanced surgical skills. With the pig in dorsal recumbency a midline incision is made from the xiphoid to the umbilicus or even more caudally. The retroperitoneal portion of the pancreatic tail is dissected free to the level of the duodenum which requires dissection and ligation of the pancreatic artery. Branches of the pancreatoduodenal artery supplying
the pancreas are ligated along the duodenum without compromising the blood supply to the intestine. The pancreatic duct is a firm, whitish structure and it is ligated when it is encountered in the caudal portion of the pancreatic attachment to the duodenum. The most difficult part of the dissection involves freeing the pancreatic body from the portal vein and cranial mesenteric vessels which it surrounds. During the dissection care should be taken to avoid contaminating the abdominal cavity with pancreatic enzymes and the surgical site should be flushed copiously with saline.

Complications of diabetes similar to those which occur in Type 2 diabetes and the cardiometabolic syndrome may be induced by feeding an atherogenic diet in addition to chemical ablation of the Beta cells. This has been performed in a variety of minipigs. Lesions associated with accelerated atherosclerosis may be seen in the vasculature within a few months. It has been demonstrated in Yucatan pigs with alloxan induced diabetes and dyslipidemia due to feeding an atherogenic diet that the development of some of the early signs of diabetic retinopathy, renal capillary basement membrane thickening, coronary artery atheroma, arterial fatty streaks and arterial intimal thickening will be present. Females are more susceptible and may develop obesity, insulin resistance, and increases in total cholesterol, triglycerides, and LDL/HDL. Hypertension may also be present. Leptin levels >2ng/ml may be an indication of clinical obesity. Some breeds of pigs, such as the wild-caught Ossabaw, may be more genetically predisposed to Type 2 diabetes and the cardiometabolic syndrome. Many of the proposed induced models have been developed in the Yucatan.

Post Procedural Maintenance
Following the induction of diabetes, long term survival is dependent upon the control of hyperglycemia; and in the case of complete pancreatectomy, the exocrine enzymes also have to be provided as a supplement. Whether the diabetes is controlled with insulin or some other therapy, such as transplantation, is dependent upon the goals of the research.

In the short term, hyperglycemia will not be fatal to the animal and they may be monitored for a few days prior to institution of an insulin protocol but generally they will die within 10 days if insulin therapy is not instituted. As a general rule swine require approximately 1.6 total units of insulin/kg, however, control must be monitored by measurement of blood glucose. Types of insulin generally used are: regular (4 hours duration), Lente (12 hours duration) and Ultralente (24 hours duration). Many protocols utilize a mixture of the various types. For example regular insulin and Lente can be given in the morning and Ultralente in the afternoon. Hypoglycemia may be fatal, consequently blood glucose
levels are generally maintained in a range of 200-300 mg/dl. If hypoglycemia occurs it may be treated by IV glucose solutions. Blood glucose levels are depressed to some degree by all anesthetics and sedatives.\textsuperscript{4,16-18} If large volumes of blood are needed then chronic catheters or vascular access ports should be implanted. Swine can be trained with food treats to allow the collection of small amounts of blood to be collected with a skin prick. Food treats with minimal caloric value, such as dog biscuits, may be used for this purpose.\textsuperscript{4,12}

Dietary therapy or manipulation may be part of the research protocol. However, if a normal swine ration is being fed then approximately 4% of the body weight of the pig is provided as a daily ration. If a total pancreatectomy has been performed, then enzymatic therapy with Viokase-V\textsuperscript{®} (Ft. Dodge Labs) should be mixed with the food to aid proper digestion. Approximately 7 gm of the enzymatic solution is sufficient to digest 2200 gm of commercial ration.\textsuperscript{4,12}

**Summary**

Miniature swine will continue to be used as a large animal model of diabetes, accelerated atherosclerosis and the related cardiometabolic syndrome. Swine are valuable as a preclinical model because of their size and shared anatomic and physiologic characteristics with humans.\textsuperscript{4} In diabetes research they offer distinct advantages in the ability to feed them test diets with a similar composition to human diets because they are true omnivores. There is also the ability to utilize them as test subjects for transplantation and xenotransplantation therapies. The similarity of their Beta cells and insulin has been known for decades because pork insulin derived from pig pancreases in slaughter houses was utilized as a standard therapy for human diabetics for many years. Refinement of the existing models should result in analogous models of all aspects of diabetes, the pre-diabetic condition and the complications of diabetes. Miniature pigs, in particular the Yucatan, will be used for the chronic studies because of the background of information that exists, their small size at maturity and their reduced growth curve when compared to farm pigs.

**References**

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