
The Science and Technology of Diagnosing Diabetes Mellitus

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The Biology of Insulin

Insulin and homeostasis

Insulin is a small hormone containing 51 amino acids, in 2 chains linked by disulphide bridges¹. It is coded for on chromosome 11 and highly conserved across species, differing only by 1 amino acid from porcine insulin.² It is synthesized in the Islets of Langerhans, pancreatic β cells. Its secretion is the dominant regulator of metabolism when in the fed state, and maintained at basal levels when in the fasted state.

Insulin is required to maintain blood glucose levels at an optimum concentration. It serves to encourage the storage of blood glucose in muscles, and also to inhibit the mobilization of amino acids, fatty acids and glycogen. Its counterpart is another hormone named glucagon, whose primary role is directly opposite that of insulin - to release stored sugars back into the bloodstream and synthesize glucose through the breakdown of glycogen.

Consequently, its secretion is mainly stimulated by increased blood glucose levels, although biochemicals such as other sugars, fatty acids, ketone bodies (from lipid breakdown) and hormones may also play a part. Obviously, physical factors such as the oral intake of glucose also stimulate high insulin levels, due to the need to remove blood sugars in the shortest possible interval. These secretion levels often peak 30 minutes after consumption of food and fall back to normal 90 minutes after, when glucose levels have returned to normal.

“Diabetes is the disease state that results from inadequate insulin action”. That is, when insulin is impaired and unable to maintain blood glucose levels, that condition is termed diabetes. This condition is often characterized by hyperglycemia, which is raised sugar levels, and inability to control glucogenesis, which also results in raised blood glucose levels. The 2 major classes of diabetes are Type 1 and Type 2. Type 1 diabetes is an autoimmune-mediated state, often present in the young, whereas Type 2 diabetes is triggered by insensitivity to insulin secretions, often presenting itself in middle-age and elderly. Previously, they were often termed as IDDM and NIDDM (Insulin Dependent Diabetes Mellitus and Non-Insulin Dependent Diabetes Mellitus respectively). However, this nomenclature was recently eliminated because they classified patients based on treatment, rather than etiology.³

Type 1 Diabetes

Type 1 diabetes is an autoimmune problem resulting from a specific attack in which the β cells are specifically destroyed by the body's own immune system. The genes responsible for type 1 diabetes are largely unknown but the single largest genetic effect stems from the major histocompatibility complex (MHC) on the short arm of chromosome 6.²

Having the genes however, does not equate to having the disease. A trigger or precipitating factor is required, though this is largely unidentified.⁴

Once the auto-immune process is set in motion, T-cell-mediated immune attacks on the β reduce the insulin output, resulting in increasing insensitivity to raised blood glucose concentrations.

Patients with Type 1 diabetes will normally present symptoms such as weight loss, severe dehydration, ketoacidosis, and possibly, coma.

Type 2 Diabetes

Type 2 diabetes is a disorder caused by insulin insensitivity to glucose, and unlike Type 1 diabetes, there is no lack of insulin in circulation. The causes and mechanisms of the disease are largely unknown, but risk factors known to be involved include obesity, ethnicity, family history, hypertension, high glycemia load diets and lack of exercise.

It is noted that insulin resistance alone cannot explain Type 2 diabetes. "Another defect superimposed on the insulin resistance may be responsible for the impaired insulin resistance"⁴

Patients with this form of diabetes often complain of blurring of vision, myopia, recurrent infections, urinary tract infections, and the like.

Complications

Further complications of diabetes include arteriosclerosis, peripheral vascular disease, coronary artery disease, cerebrovascular disease, and acute nephrotubular necrosis. While these are not covered in great detail in this report, it must be noted that it is often these complications that point to the insidious and silent onset of diabetes.

Screening & Diagnosis methods

In Type 1 Diabetes

While Type 1 diabetes does not always express itself, it is known to develop in 2% - 5% of relatives of patients⁴. Screening methods were then developed to go about detecting those at risk. These mostly involve the detection of the autoantibodies due to the nature of the disease. This is preemptive screening assuming that cases of Type 1 diabetes were previously detected in the family.

Prediction is mainly based on the presence of humeral immune markers (i.e. autoantibodies)⁵. Antibodies to IA-2A, glutamic acid dehydrogenase (GADA) and insulin have been proven useful for the radio-binding assay for this.⁶

Should the patient be unfortunate enough to be in the dark about his family history, the only other detection method are glucose loading tests, available in oral and intravenous forms. These are by no means accurate and further testing is required to differentiate between Type 1 and Type 2 diabetes.

In Type 2 Diabetes

Type 2 Diabetes can present itself in many ways. Patients normally seek diagnosis when presented with polyuria, polydipsia and weight loss.¹It may

also be detected in tandem with other illnesses, such as myocardial infarctions, strokes and infections.

For most part, we are interested in the glucose tolerance test, to see if the body responds adequately to increased glucose levels. This is because the impairment of insulin function is the cause of the problem, and should therefore be the trialed variable.

Lab measurement of Glucose

Methods of measuring glucose levels in the blood can be categorized into 2 main ones - those that utilize the reducing properties of glucose, and those that utilize enzymes.⁷

Keeping in mind that blood is a very complex substance containing many components, reagents utilized must be very specific to glucose. This however, is not possible due to the non-specific nature of reagents used and often yields results higher than their actual values, due to other impurities that may have reducing properties themselves.

Current methods of blood glucose measurement involve the use of enzymes because of their specificity. Glucose oxidase is the most commonly used enzyme in this case. Combining the enzyme and glucose yields gluconic acid and hydrogen peroxide.

Peroxidase is then used in the second part of the reaction to turn the hydrogen peroxide into water and oxygen. This oxygen is then accepted by 4 aminophenazone, an oxygen acceptor. Combined with phenol, a pink coloration appears and its intensity, when, determined by colorimetric methods at wavelength 515nm.⁸

Depending on the type of test carried out, reference ranges for glucose levels may vary. Listed below are the types of glucose tests as listed by Gail Vaughn in the textbook: Common Laboratory Tests.⁷

- 1) Fasting glucose, also known as Fasting Blood Sugar (FBS), is measured after a 12 hour fasting period from a sample of serum or plasma - 70mg/dL - 110 mg/dL.
- 2) Random glucose, though normally used in diagnosis, can be used to screen out very high glucose concentrations as are present in Type 1 diabetes. Upper limit lies at about 130mg/dL - 140 mg/dL.
- 3) 2-hour post-prandial test: Glucose sample is taken 2 hours after consumption of a meal. Values of 140mg/dL or less after 2 hours effectively rules out diabetes in most cases.⁹ The value of this kind of testing, however, is debatable because carbohydrate content may vary from meal to meal.

Glucose tolerance tests

The glucose tolerance test examines the body's ability to deal with excessive sugar levels in the bloodstream. A relatively high dose of glucose is administered either intravenously (straight into the blood stream) or orally (by way of a glucose-rich drink).

In order that the tests may be used for any diagnosis, standardization must be enforced. Notably, the following parameters must be observed:

- 1) Diet should have adequate carbohydrate intake over the prior 3 days. This is to ensure that the circulation is adequately supplied with glucose.
- 2) Non-essential medication will have to be withheld for the period of 3 days as they may interfere with insulin action. Similarly, alcohol intake is not allowed in the 3 days prior to the test as it affects the body's reaction.
- 3) Illnesses that lead on to fevers may also result in a diabetes-like response and tests should be delayed 2 more weeks. In more severe cases of illness, like myocardial infarction, burns, trauma, etc, transient hyperglycemia may occur, and a minimum of 6 weeks is required to stabilize nominal values.
- 4) 12 hours of fasting must be practiced before any testing, together with abstinence from smoking and exercise

Because glucose tolerance varies throughout the day, the test must be conducted in the morning, as tolerance significantly decreases in the afternoons. A quick sample is taken to ensure that glucose levels are not elevated because they may rise to dangerous levels on loading.

A glucose-laden drink with about 75g of glucose for non-pregnant patients is ingested orally and blood glucose measurements are taken every hour.⁸ Based on the American Diabetes Association guidelines, glucose levels of 140mg/dL - 200 mg/dL indicate pre-diabetes (impaired tolerance) and values of 200 mg/dL and above are confirmed cases of diabetes.¹⁰

Immunoassays for autoantibodies for prediction of Type 1 Diabetes

Immunoassays for the prediction of Type 1 Diabetes are not well established and methods haven't been standardized as yet¹¹. However the basic methodologies have currently reached some form, enabling limited discussion.

Radioimmunoassays are conducted using antigens that compete with our target molecules. These are tagged with radioactive portions and mixed in with antibodies. After a period of incubation, the whole mixture is centrifuged and the supernatant is separated from the product that has bonded to the wall of the container.

The sample is then assayed using a radioactive counter that counts the amount of remaining radiation. Those with radioactive portions are known to compete with the target molecules, so the higher the radioactive count, the lower the concentration of the target molecule.

In the case of diagnosing for type 1 diabetes, the following antibodies are being targeted, together with their assay specificities from a recent research paper by Petri Kulmala⁴:

- 1) Islet Cell Antibodies (ICA) - Results were expressed as Juvenile Diabetes Foundation (JDFs), with sensitivities of 100%, specificities of 98% and consistency of 98%

- 2) IA-2 antibodies (IA-2A) - Disease specificity of 97% and sensitivity of 62%
- 3) GAD Antibodies (GADA) - Sensitivity of 80% and specificity of 94%
- 4) Insulin Autoantibodies (IAA) - Sensitivity of 26% and specificity of 97%

These numbers are by no means representational of all radioimmunoassay, but provide a rough idea on how specific the assays are in picking out the intended molecules.

With testing of this sort, presence of ICA and GADA predicted insulin therapy at 6 years in 94% and 84% of patients.¹² Early diagnosis of Type 1 diabetes enables treatment to start early, and is thought to slow down the β -cell destruction.

Monitoring of Blood Glucose after diagnosis

After diagnosis, patient self-monitoring is essential for the continuation of treatment. With their own equipment, patients or their caregivers can manage blood glucose levels, and alter their diets or insulin intake as required.⁹

The most common method of doing so is by using their own reagent strips and hand-held electronic meters.

The methods of assay are essentially identical in principal to those of the labs. The reagent strips contain glucose oxidase, and produce hydrogen peroxide when mixed with glucose. This alters the pH of the test substance (in this case, blood) and provides a means by which the glucose concentration can be assayed through voltage differences.

However, because rigid test conditions cannot be enforced with the use of such meters, so they are by no means methods of diagnosis.

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