Signal transduction of insulin

Diabetes mellitus is a severe chronic disease, affecting 6–11 percent of the populations aged 30–64 and about 20 percent of those older than age 65 throughout the world. Diabetes is reaching pandemic proportions, largely owing to an increase in type II diabetes, as the lifespan is extended and more agricultural and subsistence economies adopt “westernized” lifestyles. The purpose of the seminar is to review the mechanism of insulin secretion, the major pathways of insulin-mediated signal transduction, and discuss some experimental data related to the pathogenesis of type II diabetes mellitus.

**History**

Although there may be older references in Chinese literature to a fatal wasting disease with increased urination, the first description of diabetes appears in the Ebers papyrus in 1500 BC. The term “diabetes”, coined by Demetrios of Apamaia in 200 BC, is derived from the Greek word diabeinein, meaning siphon. It was meant to capture the excessive urination and “melting down of flesh and limb into urine”. The Latin appellation “mellitus”, meaning honey, was added centuries later with the recognition that the urine of diabetics was sweet tasting. The clear distinction between diabetes mellitus and another endocrine disease associated with voluminous urination, diabetes insipidus, was made in the 18th century. The modern era, characterized by experimentation, followed. Sugar was identified in 1838 as the substance conferring the sweet taste to diabetic urine. Subsequent investigations revealed the role of the liver and pancreas, and of the pancreatic islet cells in particular, in the development of diabetes. The preparation of an active pancreatic extract and its usage for treatments of dogs, and then humans by Frederick Banting, Charles Best, JFR Macleod, and JB Collip followed. Until the first successful insulin treatment in a 14-year-old boy in 1922, the acute onset of diabetes during childhood was a death sentence. If children did not die with the initial episode of diabetic coma, they almost all perished in the next 1–2 years. With insulin therapy, the “juvenile-onset” type of diabetes was no longer acutely fatal. With longer survival, however, a variety of long-term complications appeared including blindness, renal failure, loss of legs, myocardial infarction or stroke. Diabetes had been transformed from the disease which was “short, disgusting and painful”, as described by Arateus in the 1st century, into a chronic condition that was long, disgusting, and more painful. Although much of the interest of early physicians and investigators was focused on the “juvenile-onset” form of diabetes, it is only part of the story. In the late 19th century, another form of diabetes was described by Lacereaux, which occurred later in life, mostly in the well-do, and responded to a change in the diet. He named this form “fat diabetes”, to distinguish it from the fatal, “thin diabetes” in children and young people. In the mid 1930s, Himsworth proposed the terms “insulin-sensitive” and “insulin-resistant” to designate the two different clinical presentations of diabetes. During the last decades of the 20th century, an enormous amount of data has been accumulated, which led to therapies achieving an improved control of blood glucose levels, and thus, delaying the onset of devastating long-term complications. Diabetes mellitus is now recognized as a syndrome, and comprises a heterogeneous collection of disorders, with type II diabetes, by far the most common presentation.

**Clinical diabetes**

**Definition**-Diabetes mellitus is a heterogeneous set of disorders characterized by two features: disordered metabolism, most notably hyperglycemia, and the propensity to develop specific long-term complications, such as retinopathy, nephropathy, macro-, and microangiopathy, and neuropathy. The underlying cause of all forms of diabetes is relative or absolute insulin deficiency.

**Classification**-**Type I** (formerly “insulin-dependent” or “juvenile-onset”) diabetes mellitus is caused by pancreatic β-cell destruction, often autoimmune-mediated, that
leads to loss of insulin secretion and absolute insulin deficiency. Comprises 5-10% of all cases in the diabetes syndrome. **Type II** (formerly “noninsulin-dependent” or “adult-onset”) diabetes mellitus is caused by combination of genetic and non-genetic factors that result in insulin resistance and relative insulin deficiency, insufficient to meet increased demands imposed by insulin resistance. The specific genes are not known, but are under intensive investigation. Non-genetic factors include increasing age, high-calorie intake, overweight, central adiposity, sedentary lifestyle, and low birth-weight. Comprises 90-95% of all cases. **Other specific types** of diabetes include a heterogeneous group of cases with genetic defects in insulin secretion or insulin action, as well as a large number of well-defined illnesses leading to secondary disturbances in insulin resistance, insulin secretion, or both. Only 1-2 % of all diabetes mellitus cases belong to this group. **Gestational diabetes** is defined as diabetes with onset during pregnancy. Occurs in 3-5% of all pregnancies. Usually remits after delivery, but returns later as type II diabetes in about 50% of women who had gestational diabetes.

*A brief natural history of type II diabetes mellitus*—The majority of type II diabetes occurs after age 40, with more than 50 % of the cases after age 55. The disease, however, has an insidious onset, and often goes undetected because the symptoms are either unrecognized or ignored. Type II diabetes results from the interaction of genetic and environmental factors. There is an almost 100 % concordance in identical twins, it runs through families (having one type II diabetic parent means a 2fold risk, having two diabetic parents means a 4-fold risk to develop the disease), its prevalence in ethnically diverse communities varies by the genetic background, and is extremely common in certain inbred populations. Although a genetic background for the susceptibility to type II diabetes is widely recognized, the specific genes have yet to be identified. The development of the disease is strongly influenced by environmental factors, while 50 % of Pima Indians living in a reservation in Arizona present with type II diabetes, Pima Indians in Mexico, farming and living at a subsistence level, have not developed diabetes. Similarly, the prevalence in Nauru is 40%, but the native Nauruan population did not develop diabetes until the mining of guano changed their lifestyle from subsistence fishing and farming to a sedentary lifestyle, with motorbikes, high-fat diet and obesity. Obesity, and the central (or abdominal) obesity form in particular, is also associated with type II diabetes, but usually only a small proportion of obese people contract the disease.

*Development of type II diabetes.* Insulin resistance, simply expressed as reduced sensitivity to the effects of insulin, is the most common underlying abnormality in type II diabetes, and it is detectable in people way before the onset of symptoms. The state seems to be fueled by obesity. β-Cells of the pancreas normally compensate for insulin resistance by increasing basal and postprandial insulin secretion. At some point, β-cells can no longer compensate, failing to respond appropriately to glucose. This leads to the development of glucose intolerance, and in a year 5-10% of the patients with glucose tolerance progress to diabetes mellitus, which continues to worsen as insulin resistance increases. In the late stages of the disease β-cells undergo complete failure and high doses of exogenous insulin may be required.
Insulin receptor-mediated signal transduction

Insulin receptor
The insulin receptor is a transmembrane glycoprotein, composed of two α subunits and two β subunits covalently linked through disulfide bridges to form an α\(_2\)β\(_2\) heterotetramer. The α subunit is entirely extracellular and contains the sites for insulin binding, the β subunit has a small extracellular portion, a transmembrane domain and an intracellular portion having insulin-regulated protein tyrosine kinase activity. In the absence of insulin, the α subunit suppresses the tyrosin kinase activity of the β subunit. Binding of insulin to the α subunit results in a conformational change that releases inhibitory constraints and allows tyrosine kinase activity. The receptor then undergoes a series of intramolecular transphosphorylations in which one β subunit phosphorylates tyrosine residues in the adjacent β subunit. This autophosphorylation regulates the tyrosine kinase activity of the C-terminal part and precedes the tyrosine phosphorylation of endogeneous substrates. In addition to tyrosine phosphorylation, the insulin receptor may undergo serine and threonine phosphorylation, e.g. by cAMP-dependent protein kinase, or protein kinase C. In contrast to tyrosine phosphorylation, serine phosphorylation has a negative effect on receptor tyrosine kinase activity. Serine phosphorylation has been suggested to play a role in the decreased receptor tyrosine kinase activity observed in type II diabetes, but exact details and relationships remain unclear. Counterregulatory hormones, like epinephrine may antagonize insulin action by serine phosphorylation of the receptor. Naturally occurring mutations of the insulin receptor are rare, and the phenotype, when present, shows severe insulin resistance. Some of these mutations are classified as “other types” of diabetes mellitus, but according to genomic analysis mutations in the insulin receptor do not play a role in the development of typical type II diabetes mellitus.

Insulin receptor substrates (IRS proteins)
Once activated, the insulin receptor phosphorylates a number of substrates, including members of the insulin receptor substrate family (IRS-1, IRS-2, IRS-3, IRS-4). IRS proteins recognize and bind to phosphorylated tyrosine residues in the insulin receptor and once phosphorylated by the receptor tyrosine kinase, will serve as docking proteins for signaling molecules that have SH2 (Src-homology) domains, such as PI3K (phosphatidylinositol 3 kinase), SHP2 (a tyrosine phosphatase), and Grb2 (a small adaptor molecule). The tissue distribution of the various isoforms is different, while IRS-1 and IRS-2 are ubiquitously expressed, IRS-3 is mainly restricted to the adipose tissue, and IRS-4 is most abundant in kidney and brain. Genetic polymorphisms influencing the IRS family have been described, and although none by itself is associated with the disease, certain combinations of the polymorphisms are more frequent in type II diabetics than in the normal population.

Molecular events downstream from the IRS proteins
Divergence of the insulin-signaling pathway into metabolic, growth promoting, and other biological effects probably occurs at the level of SH2 adaptor proteins that bind to phosphorylated tyrosine residues through their SH2 domains. Grb-2 is an SH2 adaptor protein that docks with IRS upon insulin stimulation and initiates processes leading to the activation of the MAP kinase cascade. This cascade mediates the mitogenic effects of insulin,
and is commonly activated by other growth factor receptors than insulin. The key element of the pathway leading to the metabolic effects of insulin is probably the activation of phosphatidylinositol-3-kinase (PI3K). PI3K is a heterodimer consisting of a regulatory subunit (p85) that associates with IRS and a catalytic subunit (p110) that phosphorylates phosphatidylinositols in the cellular membrane at position 3 in the inositol ring. Phosphatidylinositol-3,4,5-trisphosphate activates PDK1 (PI3K-dependent kinase-1) and the downstream sequence of events includes activation of protein kinase B (also named Akt), isoforms of protein kinase C (\(\xi, \gamma\)), mTOR (mammalian target of rapamycin) and P70S6 kinase. Protein kinase B is a Ser/Thr kinase with substrates including glycogen synthase kinase-3 (GSK-3), cAMP response element binding protein, and certain transcription factors. mTOR is a member of the PI3K family, and its substrates are Ser residues in proteins. mTOR increases translation via the p70S6 kinase. Collectively, these kinase cascades mediate the metabolic effects of insulin, such as translocation of GLUT4 transporters from intracellular pools to the plasma membrane, stimulation of glycogen and protein synthesis, and initiation of specific gene transcription (e.g. glucokinase). There is also a PI3K-independent pathway that mediates insulin-stimulated glucose transport.

**Insulin receptors in membrane lipid rafts- the Cbl/CAP pathway**

Lipid rafts are special regions of the plasma membrane enriched in cholesterol, sphingolipids, GPI-anchored proteins, and various signal transduction components. Insulin receptors have been detected in lipid rafts, and their activation leads to a PI3K-independent way of GLUT-4 translocation to the plasma membrane. The activated insulin receptor recruits a homodimer adapter molecule, the APS, which binds to phosphotyrosine residues on the receptor and is subsequently Tyr-phosphorylated by the receptor. The phosphorylated APS binds the Cbl/CAP complex. Cbl, a protooncogen, recognizes APS with its SH2 domain, and CAP (Cbl-associated protein) can bind to flotillin via its SoHo domain. Flotillin is a protein abundant in caveolae, and it can interact with the cortical actin filaments of the cytoskeleton. Once Cbl is Tyr-phosphorylated by the receptor, it will activate C3G (a GDP/GTP exchange factor) via Crk (an adapter molecule, complexed with C3G). The activated C3G is a GEF for the small G protein, TC10 (Rho-family), which is localized to lipid rafts, and will mediate the translocation of GLUT-4 vesicles to the plasma membrane.

**β-Cell dysfunction in type II diabetes mellitus**

Regulation of blood glucose level requires that pancreatic \(\beta\)-cells adapt their insulin secretion to the fluctuations in blood glucose concentration. Glucose equilibrates across the plasma membrane and is phosphorylated to glucose-6-phosphate by glucokinase. In \(\beta\)-cells this step determines the rate of glycolysis and the rate of pyruvate formation. Thus, when blood glucose level is high, the rate of glycolysis in the \(\beta\)-cells will increase. Compared to other cell types, an unusually high portion of pyruvate will enter the mitochondria and the citric acid cycle ensuring a significant rise in mitochondrial ATP production, and the ATP is exported to the cytosol. The increase in the ATP:ADP ratio in the cytosol causes depolarization of the plasma membrane by the closure of ATP-sensitive \(K^+\) channels. This allows the opening of voltage-sensitive \(Ca^{2+}\) channels, and
the increase in cytosolic Ca\(^{2+}\) concentration triggers the exocytosis of insulin-containing secretory granules. Some rare forms of DM are attributed to genetic defects that impair the adequate insulin response by interfering with the signaling pathways in β-cells (e.g. glucokinase, or transcription factors). In type II DM no genetic abnormalities profoundly effect β-cell function, but rather the ability of β-cells to adapt to insulin resistance that occur over lifetime. In high proportion of subjects with insulin resistance, β-cell compensation maintains normal glucose tolerance and DM may never develop. The compensatory hypersecretion of insulin in insulin-resistant states is due to an expansion of β-cell mass and alterations in the expression of key enzymes of β-cell glucose metabolism. The factors that determine the ability of the β-cell to compensate for insulin resistance are unknown. Although several mutations have been described that increase the risk of developing type II DM via impairing β-cell function, there is no clear understanding of the molecular background resulting in the manifestation of the disease.
**Recommended topics for discussion**

**Topic 1,** A brief summary of insulin effects on the metabolism and the maintenance of the blood glucose level.

A. Increased glucose uptake in skeletal muscle and adipose tissue in the postprandial state.
B. Decreased fatty acid utilization in the tissues and increased peripheral glucose oxidation.
C. Decreased hepatic glucose output

**Topic 2,** Summarize the major pathways in the signal transduction of insulin, linking certain pathways to certain biologic effects. The poster on insulin signalling can also be presented.

**Topic 3,** The role of various IRS proteins in the insulin signal transduction pathway and the development of diabetes mellitus was studied in knock out mice models. Please, discuss the following selected figures from two original papers in order to appreciate the experimental nature of biochemistry.


Fig.1f: Is the growth of mice influenced by the absence of IRS-1?

Fig.2: Are there changes in the following parameters in the IRS-1 K.O. mice? Blood glucose and insulin levels (a), glucose tolerance (b), insulin-induced hypoglycemia (c), insulin-stimulated glucose uptake in adipocytes (e). Are the IRS-1 K.O. mice diabetics or “just” insulin resistant?

Figs 3, 4. These figures present data showing that insulin stimulates the phosphorylation of the insulin receptor and the activation of PI3K even in the absence of IRS-1. The authors also identified an alternative protein transmitting the effect of insulin on PI3K, and named this protein IRS-2.

Since the IRS-1 K.O. mice were growth retarded, but not diabetic, and the study revealed the existence of IRS-2, in the next paper the role of IRS-2 was investigated.


Fig. 1e. Are the IRS-2 K.O. mice growth retarded?

Fig. 2. Are there changes in the following parameters in the IRS-2 K.O. mice? Blood glucose (a) and insulin levels (c), glucose tolerance (b), insulin-induced hypoglycemia (d), insulin-stimulated reduction in hepatic glucose output (f). Are the IRS-2 K.O. mice diabetics or “just” insulin resistant?
Fig. 3. Comparative data are presented on insulin receptor phosphorylation and PI3K activation in wild-type, IRS-1 knock out, and IRS-2 knock out mice. In IRS-1 knock outs, PI3K is efficiently activated via IRS-2, whereas PI3K is only partially activated via IRS-1 in the IRS-2 knock outs. Apparently, IRS-2 plays a major role in transmitting the metabolic effects of insulin, whereas IRS-1 is essential for normal body growth.

Fig. 4. Compare the pancreas morphology and β-cell mass in wild-type, IRS-1 K.O., and IRS-2 K.O. mice. Converge these differences with the metabolic phenotypes.

Topic 4. Pancreatic β-cell mass and function must be adequately regulated in order to maintain normal blood glucose levels. Several oral anti-diabetic drugs turned out to be modulators of the insulin secretion from β-cells, and as our molecular knowledge on β-cell function develops, new drugs shall be designed.

A. Review the mechanism of glucose-stimulated insulin secretion from β-cells.

Sulphonylurea derivatives (e.g. glibenclamid) have been used in type 2 diabetics for their stimulatory effect on insulin release. Later it was discovered, that these drugs block the ATP-sensitive K+-channels leading to membrane depolarization and insulin release.

Research data on the stimulatory role of an incretin, glucagon-like peptide-1 (GLP-1), on the insulin secretion from β-cells concluded in the invention of new anti-diabetic drugs. Exenatide activates GLP-1 receptors on b-cells, whereas sitagliptin blocks the dipeptidyl peptidase enzyme that would degrade GLP-1.

B. Adaptation of β-cells in insulin resistance (see Figure 2. in Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 2006, 444:840)

C. Loss of pancreatic β-cells, primarily because of enhanced apoptosis, in chronic hyperglycemia or hyperlipidemia. see Figs 1 and 3 in Rhodes CJ. Type 2 Diabetes—a matter of β-cell life and death. Science 2005, 307:380

Topic 5. Insulin resistance and mitochondrial dysfunction.

A. Any perturbation of mitochondrial function can result in impaired β-oxidation of fatty acids, and the accumulation of lipid metabolites, e.g. long chain acyl-CoA, diacylglycerol. These lipid metabolites can inhibit the signal transduction of insulin, which results in insulin resistance. Such a mechanism has been implicated in the reduced postprandial glucose uptake in skeletal muscle and liver in insulin resistance.

B. The glucose-stimulated insulin secretion from β-cells is based on the ATP produced in glucose metabolism, which includes ATP synthesis coupled to the building of a proton gradient across the inner mitochondrial membrane. Any perturbation of mitochondrial
function that results in impaired ATP generation from glucose in β-cells could, hence, negatively influence the glucose-stimulated insulin secretion. Uncoupling protein-2 (UCP-2) is an inner mitochondrial membrane protein that leaks protons, and in this way partially uncouples ATP synthesis from glucose oxidation leading to impaired β-cell function. (By the way, who remembers uncouplers, and UCP-1?) Hyperglycemia, and hyperlipidemia have been shown to increase UCP-2 expression, and in animal models UCP-2 deficiency improves, whereas UCP-2 over-expression impairs β-cell function. In humans, UCP-2 expression in β-cells is increased by hyperglycemia, and an UCP-2 gene polymorphism, that results in over-expression, has been linked to reduced insulin secretion and type 2 diabetes.

6. Several mechanisms have been recently described, that may link obesity and insulin resistance, type 2 diabetes at the molecular level.

Review papers:
Rosen ED, Spiegelman BM. Adipocytes as regulators of energy balance and glucose homeostasis. Nature 2006, 444:847

Free fatty acid (FFA) level in plasma is elevated in obese people, which may lead to decreased peripheral glucose disposal and impaired insulin sensitivity forcing enhanced insulin secretion. Elevated plasma FFA levels, when prolonged, can also promote β-cell apoptosis, and the loss of β-cell mass ultimately results in the manifestation of type 2 diabetes.

Besides being a fat depot, adipocytes also modulate the metabolism of the whole body by the secretion of adipokines and cytokines. A few examples:

A, Leptin secreted from adipocytes in well-fed state signals satiety in the brain, and prevents further food uptake, that would otherwise result in obesity. Genetic deficiency of leptin or the leptin receptor results in obesity (ob/ob mice, db/db mice). Leptin improves the insulin sensitivity of skeletal muscle and liver, probably via the reduction of intracellular lipid content, but there is also a cross-talk between leptin and insulin signalling.

B, Adiponectin (30 kDa) is produced exclusively in adipocytes, its plasma level is inversely related to the body mass. In obese, diabetic mice, adiponectin stimulates AMP-kinase-activated fatty acid oxidation in liver and muscle, and improves insulin sensitivity.

C, Tumor necrosis factor-a (TNF-a) is a cytokine (more detailed in next semester) produced by macrophages residing in adipose tissue. Elevated plasma levels were found obesity and insulin resistance. According to research data, in vitro, TNF-a dampens the effects of insulin. In the course of its signal transduction TNF-a may modulate insulin signalling, e.g. via an inhibitory Ser-phosphorylation of IRS-1, and this could explain, at least in part, the reduced insulin sensitivity. In animal models, blocking of TNF-a function improves insulin sensitivity (see recommended original paper, below)
D. Resistin is a small protein (12 kDa), produced probably by macrophages in adipose tissue. Its level is elevated in obesity. Resistin has diabetogenic effects: enhances hepatic glucose output and decreases glucose uptake in muscle and adipose tissue.

Recommended experiments to discuss:

Fig.1. Upon high-fat feeding, TNFα-deficient mice turn just as obese as normal ones.
Fig.2. Despite their obesity, TNFα-deficient mice are neither insulin resistant, nor diabetic: normal plasma insulin level, glucose tolerance and insulin-stimulated hypoglycemia are all preserved.