Diabetes mellitus

Practicals – experimental diabetes mellitus in laboratory animal
Definition of DM

- DM is a group of metabolic disorders characterized by hyperglycemia as a reason of impaired effect of insulin
  - absolute
  - relative
    - δ insulin resistance
    - δ impaired insulin secretion (gluco- and lipotoxicity)
- **chronic hyperglycemia** leads to cell & tissue damage (**complications**)
  - retina
  - kidney
  - nerves
Diagnosis of DM

• classical **symptoms** of diabetes + random plasma glycemia $\geq 11.1$ mmol/l
  – any time of the day
  – symptoms include polyuria, polydipsia and rapid loose of weight

• **FPG** (fasting plasma glucose) $\geq 7.0$ mmol/l
  – fasting means at least 8 h from the last meal

• **2-h PG** (postprandial glucose) $\geq 11.1$ mmol/l during oGTT
  – according to WHO standard load of 75g of glucose
Interpretation of glycemia

- **FPG:**
  - $< 6.1$ mmol/l = normal glycemia
  - $6.1 - 7.0$ mmol/l = IFG (impaired fasting glucose)
  - $\geq 7.0$ mmol/l = diabetes

- **oGTT – 2h PG:**
  - $< 7.8$ mmol/l = normal glucose tolerance
  - $7.8 - 11.1$ mmol/l = IGT (impaired glucose tolerance)
  - $\geq 11.1$ mmol/l = diabetes
Oral glucose tolerance test

<table>
<thead>
<tr>
<th>Time</th>
<th>Glycemia (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>na lačno</td>
<td>5.6</td>
</tr>
<tr>
<td>60 min</td>
<td>6.7</td>
</tr>
<tr>
<td>120 min</td>
<td>6.7</td>
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</table>

- **diabetes**
- **PGT**
- **norma**
Practicals

i.p. ANESTHESIA

1) blood sample from a tail vein
2) measurement of FPG on glucometer
3) repeated measurement of glycemia on glucometer in 30 a 90 min time intervals
4) determination of glucosuria in urine sample

1 week before 1/2 animals ALLOXAN i.v. 30 mg/kg

application of 20% glucose 1ml/100g i.p.

results:
• graph FPG - 30mPG - 90mPG
• comparison of DM x non-DM
Pathophysiology of diabetes mellitus
Regulation of glycemia

- **humoral**
  - principal
    - insulin
    - glucagon
  - auxiliary
    - glucocorticoids
    - adrenalin
    - growth hormone

- **neural**
  - sympaticus
    - hyperglycemia
  - parasympaticus
    - hypoglycemia
Mutual interchange of substrates in intermediate metabolism

**GLUCOSE**
- glucose-6-P
- pyruvate
- lactate

**ATP**

**liver, muscle**

**GLYCOGEN**
- glycogenesis, glycogenolysis

**glycolysis**

**liver, kidney, intestine**

**GLUCOSE**
- glucose-1-phosphate

**glycolysis**

**glycerol**
- glucogenic amino acids

**gluconeogenesis**

**keton bodies**
- acetyl-CoA
- citrate cycle
- respiratory chain and oxidative phosphorylation
- ATP
- H₂O

**β-oxidation**

**free fatty acids**
Question – how does glucose enter the cell???
Insulin

- preproinsulin → proinsulin → insulin + C-peptide
- exocytosis into portal circulation
  - 50% degraded during first pass through liver
- total daily production 20 - 40 U
  - 1/2 basal secretion, 1/2 stimulated
- basal secretion pulsatile
  - 5 - 15 min intervals
- stimulated – glucose, amino acids, FFA, GIT hormones
  - early phase (ready insulin)
  - late phase (synthesis de novo)
Synthesis of insulin

PREPROINSULIN (11.5 kDa)

endoplasmic reticulum

microvesicles

PROINSULIN (9 kDa)

Golgi apparatus

secretory granules

INSULIN + C-PEPTIDE

prohormon-konvertáza 2
prohormon-konvertáza 3

karboxypeptidáza

INSULIN + C-PEPTIDE
Relationship glycemia – insulin secretion

- Regulace SACHARIDY (Regulation of carbohydrates)
- GLUT-2
- Glucokinase
- Glucose-6-P
- ATP
- ATP-sensitive K⁺ channel
- Ca²⁺ channel
- Depolarization
- Ca²⁺
- Translocation and exocytosis of insulin granules

- Regulace AMINOKYSÉLINAMI (Regulation of amino acids)
- Leu
- Gln
- Arg⁺
- Lys⁺
- His⁺
Intracellular cascade of insulin receptor

- Insulin binds to the insulin receptor
- Activation of IRS (Insulin Receptor Substrate)
- Phosphorylation of IRS
- Activation of PI3-K (Phosphatidylinositol 3-Kinase)
- Formation of PIP3
- Activation of PKB (Protein Kinase B)
- Stimulation of glycogen synthesis
- Stimulation of lipogenesis
- Gene expression
- Translocation of GLUT4 to the plasma membrane
- Glucose uptake
Classification of tissues according to insulin action:

- **insulin-sensitive**
  - muscle, adipose tissue
    - facilitated diffusion by GLUT4
    - integration into cytoplasmic membrane regulated by insulin
  - liver
    - stimulation of glycogenolysis
    - inhibition of gluconeogenesis

- **insulin-non-sensitive**
  - others (incl. muscle, adipose tissue, liver)
  - transport of glucose depends on
    - concentration gradient
    - density of transporters (GLUT1-4,8-10)
    - rate of glycolysis
Diabetes mellitus

• heterogeneous syndrome characterized by hyperglycemia due to deficiency of insulin action (as a result of complete depletion or peripheral resistance)

• prevalence of DM in general population 5%, over the age of 65 already 25%
Causes of insulin deficiency

• **absolute**
  – destruction of the $\beta$-cells of the islets of Langerhan´s

• **relative**
  – insulin
    • abnormal molecule of insulin (mutation)
    • defective conversion of preproinsulin to insulin
    • circulating antibodies against insulin or receptor
  – insulin resistance in peripheral tissue
    • receptor defect
    • post-receptor defect
**Classification of DM**

<table>
<thead>
<tr>
<th>I. DIABETES MELLITUS</th>
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<tbody>
<tr>
<td>Diabetes mellitus of type 1 (T1DM)</td>
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<tr>
<td>Diabetes mellitus of type 2 (T2DM)</td>
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<tr>
<td>Gestational diabetes mellitus</td>
</tr>
<tr>
<td>Other specific types</td>
</tr>
<tr>
<td>- genetic defects of β cell function (MODY)</td>
</tr>
<tr>
<td>- genetic abnormalities of insulin receptor</td>
</tr>
<tr>
<td>- exocrine pancreas disorders</td>
</tr>
<tr>
<td>- endocrinopathies</td>
</tr>
<tr>
<td>- iatrogenic</td>
</tr>
<tr>
<td>- rare genetic syndromes</td>
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<table>
<thead>
<tr>
<th>II. IMPAIRED GLUCOSE TOLERANCE (IGT)</th>
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<tbody>
<tr>
<td>- with obesity</td>
</tr>
<tr>
<td>- without obesity</td>
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Type 1 DM (formerly IDDM)

- selective destruction of $\beta$ cells of LO in genetically predisposed individuals
  - chrom. 6 - HLA (DR3-DQ2 a DR4-DQ8), chrom. 11 - insulin gene
  - initiation by infection (viruses)
- autoimmunity mediated by T-lymphocytes (antibodies against $\beta$ cells (ICA, GAD) though)
- manifestation typically in childhood
- absolute dependence on exogenous supplementation by insulin
Type 2 DM (formerly NIDDM)

- imbalance between secretion and affect of insulin
- genetic predisposition – polygenic
  - insulin resistance
  - impairment of secretion
- clinically manifested T2DM has concomitant insulin resistance and impairment of secretion
  - due to epigenetic factors
  - typically in older adults
- 90% of subjects is obese – metabolic syndrome!!!
Insulin resistance

- physiologic amount of insulin does not cause adequate response
- compensatory hyperinsulinism
- further worsening by down-regulation of insulin receptors
Maturity-onset diabetes of the young (MODY1-6)

- group of monogenic conditions with autosomal dominant inheritance
- childhood, adolescence or early adulthood onset
- genetically determined β-cells dysfunction
  - but long-term measurable C-peptide without signs of autoimmunity
- 1% of diabetic patients

- two subgroups
  - mutations in glucokinase (MODY2)
    - glucokinase = glucose sensor (production and releasing of insulin is slowing)
    - mild form without considerable risk of complications
  - mutations in the genes encoding transcription factors (remaining 5 types)
    - severe β-cells defects progressively leading to diabetes with serious complications
    - Affected glucose-stimulated production and release of insulin and also proliferation and differentiation of β-cells
## Main characteristics of T1DM and T2DM and MODY

<table>
<thead>
<tr>
<th></th>
<th>T1DM</th>
<th>T2DM</th>
<th>MODY</th>
</tr>
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<tbody>
<tr>
<td>Onset</td>
<td>childhood</td>
<td>adults</td>
<td>childhood</td>
</tr>
<tr>
<td>Genetic disposition</td>
<td>yes (oligogenic)</td>
<td>yes (polygenic)</td>
<td>yes (monogenic)</td>
</tr>
<tr>
<td>Clinical manifestation</td>
<td>often acute</td>
<td>mild or none</td>
<td>mild</td>
</tr>
<tr>
<td>Autoimmunity</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>no</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Dependence on insulin</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Obesity</td>
<td>no</td>
<td>yes</td>
<td>no</td>
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Clinical presentation of manifest DM

- due to the increase of blood osmolality, osmotic diuresis and dehydration
  - classical
    - polyuria
    - thirst
    - polydipsia
    - weight loss
    - temporary impairment of visus
    - cutaneous infections
  - acute
    - hyperglycemic coma
      - ketoacidotic
      - non-ketoticidotic
    - hyperosmolar nonketoacidotic hyperglycemia
    - lactate acidosis
Complications of DM

- **microvascular**
  - diabetic retinopathy
  - diabetic nephropathy
  - diabetic neuropathy (sensoric, motoric, autonomic)

- **macrovascular**
  - atherosclerosis (CAD, peripheral and cerebrovascular vascular disease)

- **combined**
  - diabetic foot (ulcerations, amputations and Charcot´s joint)

- **others**
  - periodontitis
  - cataract
  - glaucoma