New approaches for treatment of type 1 diabetes

October 20, 2008
Regulation of blood sugar by the pancreatic “glucostat”

Glycolytic rate in pancreas and liver cells directly reflects blood glucose level
OTHER CELLS CANNOT MEASURE BLOOD GLUCOSE!

<table>
<thead>
<tr>
<th>Tissue distribution:</th>
<th>Hexokinase</th>
<th>Glucokinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_m$</td>
<td>Most</td>
<td>Liver and β cells</td>
</tr>
<tr>
<td>$V_{max}$</td>
<td>Low (0.1 mM)</td>
<td>High (10 mM)</td>
</tr>
<tr>
<td>Inhibition by G6P</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Insulin also regulates glucose uptake in muscle and fat cells

When insulin levels increase, glucose transporters move from cell membrane to intracellular storage pool, where they can be recycled.

Glucose transporters increase insulin-mediated uptake of glucose into cell.
Human metabolism distributed among different organs

The well-fed state

The fasting state
Insulin signals the well-fed state

Increases:
- glucose uptake by muscle and adipose tissue
- glycolysis
- glycogen synthesis
- triacylglycerol synthesis
- synthesis of DNA, RNA, protein

Decreases:
- gluconeogenesis
- fatty acid oxidation
- protein degradation
Stages in glucose homeostasis

Diabetes: Tissues think that they are starving even though blood sugar remains high.
Diabetes mellitus: syndromes characterized by elevated blood glucose

**Type 1** (juvenile-onset diabetes, insulin-dependent diabetes)
Autoimmune destruction of pancreatic $\beta$ cells resulting in insulin deficiency. Sudden or gradual onset when $>90\%$ of $\beta$ cells are lost.

**Type 2** (adult-onset diabetes, non-insulin-dependent diabetes)
90-95\% of diabetes cases, $\sim5\%$ of adults in industrialized societies. Insulin resistance and/or abnormal insulin secretion.


**Inherited single-gene defects** (rare) can cause syndromes resembling type 2. Examples: glucokinase deficiency (heterozygous), some dominant negative PPAR$\gamma$ mutations, some $K^+(ATP)$ channel mutations.
Chronic hyperglycemia causes severe diabetic complications

Glucose excreted in urine, massive increase in urine production, dehydration
Stress on kidneys can result in renal failure
High blood pressure due to increase in blood osmolytes can lead to retinopathy
Frequent fungal infections due to low pH environment
Swelling of tissues that convert glucose into sorbitol, including lens and nerve, and microvascular pathologies resulting in peripheral neuropathies and retinal damage
Non-enzymatic glycosylation of blood vessel lining promotes atherosclerosis, stroke, and cellulitis
Hyperlipidemia
Ketoacidosis (primarily in type 1)
Tests for glucose monitoring

A1c test for glycated hemoglobin
Monitors average blood glucose over the past few weeks or months (note RBC lifetime 120 days)
Normal around 5%
Diabetic over 6% (not a diagnosis)
Successful treatment <7% or <6.5%

Type 1 self-monitoring requires 3-10 blood samples per day
Continuous blood glucose monitors require an implant under the skin (measure glucose in interstitial fluid); used with insulin pumps
Consequences of diabetic hyperglycemia

- Osmotic stress
- Peripheral neuropathy

Effects on endothelial cells

Effects on blood flow, inflammation

Buildup of glycosylated proteins in retinal blood vessels, kidney glomeruli

Origins of diabetes

Type I: Destruction of insulin-producing pancreatic islet cells (autoimmune, can be triggered by viral infection)
Type II: Related to overall metabolic state (positive feedback loop?)

Diabetes = fasting glucose >126 mg/dL, twice in a row, and/or glucose spikes >200 mg/dL
β cell mass increases in response to demand/resistance of tissues

Mouse models for insulin resistance
IR = insulin receptor
IRS1 = insulin receptor substrate 1

Kulkarni et al., 2003, Diabetes 52: 1528
Insulin in type 2 diabetes:

Increased demand for insulin causes β cell compensation
β cells secrete more insulin

Chronic hyperinsulinemia causes:
  - Narrowing of blood vessels, hypertension
  - Risk of congestive heart failure
  - Neovascularization, bleeds in the eye

Insulin demand continues to increase

“Glucose toxicity” causes decrease in insulin secretion

Eventually, β cell damage and death
  - Role for ER stress
  - Associated with amyloid formation
  - Mice overexpressing IAPP (aka amylin) develop diabetes: cause or consequence?

pink-amyloid
brown-islet cells
Distinct mechanisms of β-cell death in type 1 vs. type 2 diabetes

**T1D**
- Activated macrophages
- Cytokines
  - NO
  - FasL
- Chemokines
- β-cell
  - ↑ Gene networks
  - ↑ iNOS
  - ↑ Caspase 3
  - Apoptosis

**T2D**
- High glucose
- FFA
- Increased physiologic turnover (?)
- Mechanisms (?)
- β-cell
- Apoptosis
- Necrosis
  - Attraction of non-activated scavenger macrophages
  - β-cell removal
  - No or minimal inflammation
Genetic predispositions to type 1

HLA region on chromosome 6p21 accounts for about half of familial clustering including both PREDISPOSING and PROTECTIVE haplotypes

Mostly HLA-DR and HLA-DQ; MHC II

Transgenic mice: tend toward failure of self-tolerance to insulin and glutamic acid decarboxylase (putative autoantigens)

Insulin gene variable number of tandem repeats upstream (each 14-15 bp)

Class I alleles (26-63 repeats) predispose
Class III alleles (140-210 repeats) protective, dominant
Class III alleles are associated with higher levels of insulin mRNA expression in the thymus (!)

Genes associated with T-cell activation

  Lymphoid tyrosine phosphatase
  CTLA-4
  Interleukin-2 receptor

Others including SUMO-4
Self-tolerance

Both central and peripheral mechanisms contribute

Cytokines released by dying β-cells may trigger a positive feedback loop
Type 1 diabetes is treated with insulin

- 1921 - Purified from cow pancreas (Banting and Best)
- 1922 - First used in human patients
- 1978 - Recombinant form produced in bacteria (Genentech)
- 1982 - First recombinant drug approved by FDA

Delivery mechanisms: Frequent injections - basal and prandial (problems with compliance, control)

Insulin pumps (programmable)

Limited use in early type 2 diabetes (may be used for later salvage)

Note: made by processing a pre-protein; cleavage generates “C-peptide” (used to measure insulin synthesis rates)
### Therapeutic agents for type 2 - 2000

<table>
<thead>
<tr>
<th>Drug class</th>
<th>Molecular target</th>
<th>Site(s) of action</th>
<th>Adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>Insulin receptor</td>
<td>Liver, muscle, fat</td>
<td>Hypoglycaemia, weight gain</td>
</tr>
<tr>
<td>Sulphonylureas (e.g. glibenclamide) plus nateglinide and repaglinide</td>
<td>SU receptor/ K⁺ ATP channel</td>
<td>Pancreatic β-cell</td>
<td>Hypoglycaemia, weight gain</td>
</tr>
<tr>
<td>Metformin — biguanides</td>
<td>Unknown</td>
<td>Liver (muscle)</td>
<td>Gastrointestinal disturbances, lactic acidosis</td>
</tr>
<tr>
<td>Acarbose</td>
<td>α-glucosidase</td>
<td>Intestine</td>
<td>Gastrointestinal disturbances</td>
</tr>
<tr>
<td>Pioglitazone, rosiglitazone (thiazolidinediones)</td>
<td>PPARγ</td>
<td>Fat, muscle, liver</td>
<td>Weight gain, oedema, anaemia</td>
</tr>
</tbody>
</table>

- Increases insulin secretion
- Inhibits gluconeogenesis
- Inhibits carbohydrate uptake
- Increases insulin sensitivity

Note range of target tissues…
Therapeutic agents for type 2 - 2007

<table>
<thead>
<tr>
<th>Medication*</th>
<th>Route of Administration</th>
<th>Year of Introduction or FDA Approval</th>
<th>Efficacy as Monotherapy, Measured as a Reduction in the Glycated Hemoglobin Concentration percentage points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>Parenteral</td>
<td>1921</td>
<td>≥2.5</td>
</tr>
<tr>
<td>Inhaled insulin</td>
<td>Pulmonary</td>
<td>2006</td>
<td>1.5</td>
</tr>
<tr>
<td>Sulfonylureas</td>
<td>Oral</td>
<td>1946</td>
<td>1.5</td>
</tr>
<tr>
<td>Biguanides</td>
<td>Oral</td>
<td>1957</td>
<td>1.5</td>
</tr>
<tr>
<td>Metformin†</td>
<td>Oral</td>
<td>1995</td>
<td>1.5</td>
</tr>
<tr>
<td>Alpha-glycosidase inhibitors</td>
<td>Oral</td>
<td>1995</td>
<td>0.5–0.8</td>
</tr>
<tr>
<td>Thiazolidinediones</td>
<td>Oral</td>
<td></td>
<td>0.8–1.0</td>
</tr>
<tr>
<td>Troglitazone‡</td>
<td>Oral</td>
<td>1997</td>
<td></td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td>Oral</td>
<td>1999</td>
<td></td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>Oral</td>
<td>1999</td>
<td></td>
</tr>
<tr>
<td>Glinides</td>
<td>Oral</td>
<td>1997</td>
<td>1.0–1.5</td>
</tr>
<tr>
<td>GLP analogues</td>
<td>Parenteral</td>
<td>2005</td>
<td>0.6</td>
</tr>
<tr>
<td>Amylin analogues</td>
<td>Parenteral</td>
<td>2005</td>
<td>0.6</td>
</tr>
<tr>
<td>DPP-IV inhibitors</td>
<td>Oral</td>
<td>2006</td>
<td>0.5–0.9</td>
</tr>
</tbody>
</table>

* GLP denotes glucagon-like peptide, and DPP-IV dipeptidyl peptidase IV.
† Metformin has been available in other countries since 1957 but was approved in the United States in 1995.
‡ Troglitazone was approved in 1997 but was withdrawn from the market in 2000 because of hepatotoxicity.
Treatment recommendations, 2007

A1C = glycated hemoglobin measured by HPLC

Diagnosis

Lifestyle Intervention + Metformin

A1C ≥ 7%

Yes*  No

Add Basal Insulin*
   - Most effective

A1C ≥ 7%

No  Yes*

Add Sulfonylurea*
   - Least expensive

A1C ≥ 7%

Yes*  No

Add Glitazone*
   - No hypoglycemia

A1C ≥ 7%

Yes*

Add Basal Insulin*

A1C ≥ 7%

No  Yes*

Add Sulfonylurea*

A1C ≥ 7%

Yes*

Add Basal or Intensify Insulin*

Intensive insulin + Metformin +/- Glitazone

American Diabetes Association
What about new therapies for type 1?

Approach #1: replace β-cells

Pancreas transplant or islet transplant (Edmonton protocol)

Problem: immunosuppressive drugs (glucocorticoids) tend to cause peripheral insulin resistance; unusual immunosuppressive protocol required

Patients tend to lose insulin independence again after about 2 years (note that underlying autoimmune response has not been addressed except by general immunosuppression)

Shapiro et al., 2000, NEJM 243:230
Shapiro et al., 2006, NEJM 355: 1318
On the horizon: β-cell regeneration?

Possibilities:

The only mode of β-cell regeneration that is known to occur normally in adult animals is replication of preexisting β-cells; no progenitors or stem cells are known

Paper #1: Zhou et al. report TRANSDIFFERENTIATION of pancreatic exocrine cells into insulin-secreting “β-like” cells by expression of three transcription factors
What about new therapies for type 1?

Approach #2: interfere with autoimmunity

Note β-cell loss is gradual; early intervention may be an effective prevention

Epidemiology: incidence varies in populations from 1-40/100,000; frequency is increasing ~3%/year, particularly among the very young

Immunization for tolerance against autoantigens

Insulin, GAD

Immunomodulation

General suppressors for autoimmune diseases, including cytokine blockers

Bollyky et al., 2008, Mt. Sinai J. Med. 75: 385
Papers for Wednesday:

**Paper 1:** Zhou, Q. et al. (2008). *In vivo* reprogramming of adult pancreatic exocrine cells to β-cells. *Nature.* **455:** 627-632