RESISTENSI INSULIN:
DEFINISI, MEKANISME PADA
DIABETES DAN PEMERIKSAAN
LABORATORIUMNYA

A.A. WIRA DEWI LESTARI

BAGIAN PATOLOGI KLINIK FK UNUD / INSTALASI
LABORATORIUM PATOLOGI KLINIK RSUP SANGLAH
DENPASAR
OUTLINE

• INTRODUCTION
• INSULIN CHEMISTRY, BIOSYNTHESIS, METABOLISM
• GLUCOSE TRANSPORT MECHANISM AND METABOLIC EFFECT OF INSULIN
• INSULIN RESISTANCE AND ASSOCIATED CONDITIONS
• LABORATORY TESTING FOR INSULIN RESISTANCE
Diabetes mellitus:
a heterogeneous disorder
defined by the presence of hyperglycemia
Hyperglycemia: functional deficiency of insulin action, due to:

1. Decreased in insulin secretion
2. Decreased response to insulin by target tissues (insulin resistance)
3. Increase counterregulatory hormones (oppose effects insulin)

10% of cases: type 1 (autoimmune B cell destruction)
90% of cases: type 2 (has a stronger genetic component, associated with resistance to the effects of insulin at its sites of action and, 80% of cases associated with obesity)

Primary lesi:

- Insulin resistance → exhausted
- Hyperinsulinemia → insulin resistance (down regulated insulin receptors number)
- Impaired early secretion of insulin → insulin resistance (hiperglikemia, hiperinsulinemia)

*Insulin resistance: hallmark of this disorder*
### Diabetes in clinical reality – Global

<table>
<thead>
<tr>
<th>Ranking</th>
<th>Country</th>
<th>People with diabetes (millions)</th>
<th>Country</th>
<th>People with diabetes (millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>India</td>
<td>31.7</td>
<td>India</td>
<td>79.4</td>
</tr>
<tr>
<td>2</td>
<td>China</td>
<td>20.8</td>
<td>China</td>
<td>42.3</td>
</tr>
<tr>
<td>3</td>
<td>US</td>
<td>17.7</td>
<td>US</td>
<td>30.3</td>
</tr>
<tr>
<td>4</td>
<td>Indonesia</td>
<td>8.4</td>
<td>Indonesia</td>
<td>21.3</td>
</tr>
<tr>
<td>5</td>
<td>Japan</td>
<td>6.8</td>
<td>Pakistan</td>
<td>13.9</td>
</tr>
<tr>
<td>6</td>
<td>Pakistan</td>
<td>5.2</td>
<td>Brazil</td>
<td>11.3</td>
</tr>
<tr>
<td>7</td>
<td>Russia</td>
<td>4.6</td>
<td>Bangladesh</td>
<td>11.1</td>
</tr>
<tr>
<td>8</td>
<td>Brazil</td>
<td>4.6</td>
<td>Japan</td>
<td>8.9</td>
</tr>
<tr>
<td>9</td>
<td>Italy</td>
<td>4.3</td>
<td>Philippines</td>
<td>7.8</td>
</tr>
<tr>
<td>10</td>
<td>Bangladesh</td>
<td>3.2</td>
<td>Egypt</td>
<td>6.7</td>
</tr>
</tbody>
</table>
Global diabetes epidemic

Number of people with diabetes worldwide (millions)

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of People (in millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>194</td>
</tr>
<tr>
<td>2025</td>
<td>333</td>
</tr>
</tbody>
</table>

72% increase
Cardiometabolic Risk (CMR)

Obesity, Metabolic Syndrome, and Type 2 Diabetes


IR and β-cell dysfunction are fundamental to Type 2 diabetes

INSULIN CHEMISTRY, BIOSYNTHESIS, AND METABOLISM
CHEMISTRY

- Is protein hormone, stored in beta-cell of pancreas as crystalline form (+Zn)
- Consist of 2 chains $\alpha$ and $\beta$ chain connected by disulfide bridge. In alpha chain there is intra disulfide bridge between two cysteine aa (6 and 11)
- Digestion of insulin by proteolytic enzyme inactivate the hormone, and for this reason it can’t be given orally
BIOSYNTHESIS

- Human insulin gene located on the short arm of chromosome 11.

- A precursor molecule, preproinsulin, a peptide of MW 11,500 is translated from preproinsulin mRNA in Endoplasmic Reticulum pancreatic beta cell.

- Microsomal enzyme cleave preproinsulin to proinsulin (MW 9000) immediately after synthesis.
• Proinsulin is transported to Golgi apparatus, where packaging into secretory granule.

• Maturation of the secretory granule is associated to conversion of proinsulin to insulin and C-peptide

• Normal mature secretory granule contain insulin and C-peptide in equimolar amounts.
• Insuline and C-peptide are then released from cells.
• C-peptide is released in an amount equimolar to insulin.
• Half life in plasma 3 - 5 minutes. Its metabolize fastly
• Liver is the main organ for insuline metabolism (50%). The others kidney and placenta.
Figure 42–12. Structure of human proinsulin. Insulin and C-peptide molecules are connected at two sites by dipeptide links. An initial cleavage by a trypsin-like enzyme (open arrows) followed by several cleavages by a carboxypeptidase-like enzyme (solid arrows) results in the production of the heterodimeric (AB) insulin molecule (light blue) and the C-peptide.
Insuline secretion stimulation

- Glucose stimulate insulin secretion and suppress glucagon secretion.

- Initiate by ATP/ADP ratio within the cell ➔ close the membrane ATP-sensitive K channel. ➔ depolarisation of cell ➔ voltage change ➔ open the Ca channel. ➔ entry Ca-ion ➔ stimulate first short phase of insulin secretion.

- Increase concentration of long chain Acetyl-CoA mol: The second, more prolonged phase
Biphasic Insuline secretion

![Graph showing biphasic insulin secretion](image)
Homeostatic regulation of blood glucose

Blood glucose levels are not fixed. For example, they rise after a meal, and then return to “pre-meal” values later.

A meal includes chemical energy (including sugar) beyond immediate need. That excess is not discarded; its saved for later use. There’s a regulated process to do this. We’ll be focusing on glucose exclusively.
GLUCOSE TRANSPORT MECHANISM AND METABOLIC EFFECT OF INSULIN
Insulin receptor & action

- Insulin receptor is the members of the growth factor family, are membrane glycoprotein composed of two protein sub unit encoded by a single gene.

- The larger sub unit (alpha) MW 135,000 D resides entirely extracellularly, where its bind insulin mol.

- The alpha sub unit connected by disulfide linkage to the smaller beta subunit MW 95,000 D. This sub unit cross the membrane and its cytoplasmic domain contain a tyrosine kinase activity that initiates intra cellular signaling pathways.
# Tissue-specific glucose transporters

<table>
<thead>
<tr>
<th>Name</th>
<th>Tissue</th>
<th>Km</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT1</td>
<td>RBC, most others</td>
<td>Medium (5mM)</td>
<td>Basal glucose uptake</td>
</tr>
<tr>
<td>GLUT2</td>
<td>Liver, pancreatic β-cells</td>
<td>High (7-20mM)</td>
<td>Allows equilibration with blood glucose</td>
</tr>
<tr>
<td>GLUT3</td>
<td>Brain</td>
<td>Low (1mM)</td>
<td>Constant uptake</td>
</tr>
<tr>
<td>GLUT4</td>
<td>Muscle, fat</td>
<td>Medium (5 mM)</td>
<td>Insulin-regulated uptake</td>
</tr>
</tbody>
</table>

Facilitated transport: glucose moves down concentration gradient
Glucose transport mechanism by insulin signaling, Depres 1999
Insulin effects

1. PARACRINE Effect
   First target cells reached by insulin is alpha cells of Langerhans islet ➔ inhibit glucagon secretion.

2. ENDOCRINE Effect
   Liver, Muscle, adipose tissue
Metabolic effect of insulin

In the liver:

- **promote anabolism**
- **promote glicogenesis and glicogen storage**
- **suppress lipolysis and promote synt of protein and lipogenesis**
- **Inhibit gluconeogenesis and promote glycolisis.**

- **Inhibited catabolism**
- **Inhibiting hepatic glicogenolysis, ketogenesis and gluconeogenesis.**
• **In adipose tissue** insulin stimulate triglyceride synthesis and storage, by induce production of lipoprotein lipase, increase glucose transport, and inhibit intracellular lipolysis

• **In muscle** stimulate protein synthesis, by increasing asam amino transport, promote glikogenesis
**Biological Action of Insulin**

- **Vasodilatation**
  - ↑ NO release
  - ↑ eNOS expression

- **Platelet inhibition**
  - ↑ NO release in platelets
  - ↑ cAMP

- **Anti-oxidant**
  - ↓ ROS generation

- **Anti-inflammatory**
  - ↓ NFκB, ↑ IkB
  - ↓ MCP
  - ↓ ICAM-1
  - ↓ CRP

- **Anti-thrombotic**
  - ↓ TF

- **Profibrinolytic**
  - ↓ PAI-1

- **Cardio-protective**
  - Animals, human

- **Anti-atherosclerotic**
  - ApoE null mouse
  - IRS-1 null mouse
  - IRS-2 null mouse

- **Anti-apoptotic**
  - Heart, other tissues

INSULIN RESISTANCE (IR) AND ASSOCIATED CONDITIONS
• IR: exist any time a normal amount of insulin produce a less than normal biologic response. Inadequate tissue response to insuline hormon (decreased insulin sensitivity and or decreased responsiveness)
Decreased insulin sensitivity and responsiveness, Joslin 2005
Insulin Resistance: Associated Conditions

- Type 2 diabetes
- Atherosclerosis
- Hypertension
- Impaired glucose tolerance
- Obesity (central)
- Dyslipidemia
- Polycystic ovary disease
- Decreased fibrinolytic activity
- Acanthosis nigricans
- Hyperuricemia

Adapted from: Overview, Development, Methods
The mechanisms responsible for insulin resistance syndromes include:

* Genetic or primary target cell defects (Defect in insulin receptor expression or sequence. Rare, but represents severe form)
* Autoantibodies to insulin
* Accelerated insulin receptor degradation/Insulin receptor downregulation
In type 2 Diabetes, defects at multiple level:

- Decreases in receptor concentration
- Decrease in receptor kinase activity
- Decrease in concentration and phosphorylation of IRS-1 and IRS-2
- Decrease in PI 3-kinase activity
- Decrease in glucose-transporter translocation
- Defects in activity of intracellular enzymes

Chronic hyperinsulinemia on vasculature
## Site of IR

<table>
<thead>
<tr>
<th>Site of rest.</th>
<th>Poss. defect</th>
<th>Role in DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prereceptor</td>
<td>Ins.receptor.antibody</td>
<td>rare.</td>
</tr>
<tr>
<td>Receptor</td>
<td>Decreased number or affinity of ins reseptor</td>
<td>not important in DM</td>
</tr>
<tr>
<td>Post receptor</td>
<td>Defect in signal Reduce IRS-1 Decrease pyruvat kinase or glycogen syntase</td>
<td>most probable source of IR</td>
</tr>
<tr>
<td>GLUT</td>
<td>Defective translocation of GLUT to cell membrane</td>
<td>Important</td>
</tr>
</tbody>
</table>
Insulin resistance mechanism in obesity, Depres 1999
Central role of FFA in insulin resistance
Tissue insulin resistance
A unifying hypothesis of type 2 diabetes
Insulin Resistance in:

1. Muscle: increased accumulation of fat and secondary insulin resistance, hypertriglyceridemia, and increased levels of free fatty acid
2. In liver: increased hepatic glucose output
3. In Brain: Increase in appetite, more obesity and further defects in hepatic glucose output
4. In β-cells: defects in glucose sensing and thereby to relative insulin deficiency.

All defects associated with type 2 diabetes
LABORATORY TESTING FOR INSULIN RESISTANCE
Gold standard: 
*hyperinsulinemic -euglycemic clamp* → *glucose clamp technique*

Estimate of insulin action based on the ability of insulin to limit the increase in plasma glucose concentration in response to continuous glucose infusion.

But: impractical, expensive, time-consuming and labor intensive.
Variety methods of varying complexity in estimate IR (own advantages and disadvantages).

Homeostasis Model Assessment (HOMA-IR model)
First described in 1985 by Matthews, strong correlation with glucose clamp technique
HOMA-IR

- **Fasting insulin (μU/mL) x Fasting glucose (mmol/L)**
  
  22.5

- HOMA-IR normal person without metabolic disorders and without obesity: 2.77

- **Low HOMA-IR**
  
  = high insulin sensitivity
  = low insulin resistance

- **High HOMA-IR**
  
  = high insulin resistance
  = prediabetic state
Computational Methods Are Significant Determinants of the Associations and Definitions of Insulin Resistance Using the Homeostasis Model Assessment in Women of Reproductive Age

Fatma H. Safar,¹ Olusegun A. Mojiminiyi,¹* Hazem M. Al-Rumaih,² and Michael F. Diejomaoh³
Table 4. Binary logistic regression analyses showing risk associations of insulin resistance (HOMA1-IR and HOMA2-IR) with PCOS, MS, and hyperandrogenism.

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th></th>
<th>Metabolic syndrome</th>
<th></th>
<th>Hyperandrogenism</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>P</td>
<td>OR</td>
<td>95% CI</td>
<td>P</td>
</tr>
<tr>
<td>HOMA1-IR</td>
<td>1.49</td>
<td>1.11–1.98</td>
<td>0.007</td>
<td>1.61</td>
<td>1.31–1.98</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HOMA1-IR adjusted for WC</td>
<td>1.49</td>
<td>1.08–2.06</td>
<td>0.016</td>
<td>1.37</td>
<td>1.12–1.69</td>
<td>0.003</td>
</tr>
<tr>
<td>HOMA1-IR ≥2.9</td>
<td>3.71</td>
<td>1.42–9.69</td>
<td>0.007</td>
<td>5.70</td>
<td>2.86–11.36</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HOMA1-IR ≥2.9 adjusted for WC</td>
<td>3.33</td>
<td>1.12–9.87</td>
<td>0.030</td>
<td>2.71</td>
<td>1.22–6.03</td>
<td>0.015</td>
</tr>
<tr>
<td>HOMA2-IR</td>
<td>2.61</td>
<td>1.35–4.86</td>
<td>0.004</td>
<td>2.07</td>
<td>1.42–3.02</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HOMA2-IR adjusted for WC</td>
<td>2.60</td>
<td>1.30–5.18</td>
<td>0.007</td>
<td>1.55</td>
<td>1.04–2.32</td>
<td>0.031</td>
</tr>
<tr>
<td>HOMA2-IR ≥1.7</td>
<td>3.49</td>
<td>1.32–9.23</td>
<td>0.012</td>
<td>4.24</td>
<td>2.10–8.55</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HOMA2-IR ≥1.7 adjusted for WC</td>
<td>3.33</td>
<td>1.12–9.90</td>
<td>0.031</td>
<td>2.23</td>
<td>1.05–5.13</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Distribution of Fasting Plasma Insulin, Free Fatty Acids, and Glucose Concentrations and of Homeostasis Model Assessment of Insulin Resistance in a Representative Sample of Quebec Children and Adolescents


Biomarkers in Fasting Serum to Estimate Glucose Tolerance, Insulin Sensitivity, and Insulin Secretion

Allison B. Goldfine, Robert W. Gerwien, Janice A. Kolberg, Sheila O'Shea, Sarah Hamren, Glenn P. Hein, Xiaomei M. Xu, and Mary Elizabeth Patti
Table 3. Models for the prediction of physiologic indicators of diabetes based on biomarkers measured in fasting blood samples.

<table>
<thead>
<tr>
<th>Model components^a</th>
<th>Estimate^b</th>
<th>Standard error</th>
<th>P^c</th>
<th>Multiple R^2</th>
<th>Bootstrap R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin sensitivity (all markers)</td>
<td>0.91</td>
<td>0.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>−0.646</td>
<td>0.2231</td>
<td>0.0048</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>−0.852</td>
<td>0.0413</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fas ligand</td>
<td>−0.011</td>
<td>0.0051</td>
<td>0.0303</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complement C3</td>
<td>−0.0001</td>
<td>0.00004</td>
<td>0.0073</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAI-1</td>
<td>−0.122</td>
<td>0.0535</td>
<td>0.0248</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin sensitivity (excluding glycemic markers and insulin)</td>
<td>0.62</td>
<td>0.58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>0.239</td>
<td>0.0453</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin</td>
<td>−0.171</td>
<td>0.0359</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAI-1</td>
<td>−0.220</td>
<td>0.0813</td>
<td>0.0083</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPT</td>
<td>−0.291</td>
<td>0.0856</td>
<td>0.0010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>−0.223</td>
<td>0.0801</td>
<td>0.0067</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Insulin Testing

Metode: Chemiluminescent (sandwich)
Insulin, a biotinylated monoclonal insulin-specific antibody, and a monoclonal insulin-specific antibody labeled with a ruthenium complex

Total duration assay: 18 minutes

Sample: serum, Li-heparin, K3-EDTA, and sodium citrate plasma, avoid hemolysis
Sample stability: room temperature 8 h; 2-8°C 24 h; <= -20°C 6 M
Reference range: 6 – 26 μU/mL or 43 – 186 pmol/L  
newborn 3 – 20 μU/mL  
critical values > 30 μU/mL

- Drugs that may cause increased insulin levels: corticosteroids. Levodopa, oral contraceptives
- In the second to third trimester of pregnancy, there is a relative insulin resistance with a progressive decrease of plasma glucose and immunoreactive insulin
Toward Standardization of Insulin Immunoassays

W. Greg Miller,¹* Linda M. Thienpont,² Kathleen Van Uytfanghe,² Penelope M. Clark,³ Patrik Lindstedt,⁴ Göran Nilsson,⁵ and Michael W. Steffes,⁶ for the Insulin Standardization Work Group

Pilot Study for the Standardization of Insulin Immunoassays with Isotope Dilution–Liquid Chromatography/Tandem Mass Spectrometry

Diego Rodríguez-Cabaleiro,¹ Kathleen Van Uytfanghe,¹ Veronique Stove,² Tom Fiers,² and Linda M. Thienpont¹*
Table 1. Error components and their possible sources.

<table>
<thead>
<tr>
<th>Error component</th>
<th>Notation</th>
<th>Possible sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error component between consecutive measurements within runs</td>
<td>e</td>
<td>1. Variation in performance conditions within runs</td>
</tr>
<tr>
<td>Error component between different positions within a run</td>
<td>c</td>
<td>2. Trends in performance conditions within runs (position effects)</td>
</tr>
<tr>
<td>Sample-specific error</td>
<td>d</td>
<td>3. Differences in influence quantities between native samples</td>
</tr>
<tr>
<td>Error component between runs (may be a function of the concentration $\mu$)</td>
<td>$b(m)$</td>
<td>4. Random error in the estimation of the calibration curve (due to the variation within runs)</td>
</tr>
<tr>
<td>Common systematic error component (may be a function of the concentration)</td>
<td>$\delta(m)$</td>
<td>5. Interaction between performance conditions and calibrator properties</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6. Error in the assigned value of the reference material used for preparation of calibrators</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7. Variation between vials of reference material</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8. Errors in the preparation of the calibrators</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9. Unsuitable model for the calibration curve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10. Unsuitable concentration levels of the calibrators</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11. Noncommutable calibrators (differences in influence quantities between calibrators and native samples)</td>
</tr>
<tr>
<td>Manufacturer</td>
<td>Slope (SE)</td>
<td>Intercept (SE), mIU/L</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>Abbott</td>
<td>1.08 (0.02)</td>
<td>−0.33 (0.21)</td>
</tr>
<tr>
<td>Beckman</td>
<td>0.84 (0.01)</td>
<td>−0.20 (0.12)</td>
</tr>
<tr>
<td>DPC</td>
<td>1.27 (0.07)</td>
<td>+0.74 (1.28)</td>
</tr>
<tr>
<td>Roche</td>
<td>1.21 (0.02)</td>
<td>−0.33 (0.31)</td>
</tr>
</tbody>
</table>

*a* The slopes and intercepts of the regression equations were derived by weighted Deming regression analysis.
Insulin resistance: hallmark of type 2 Diabetes melitus

- Insulin is a protein hormone; its secretion is stimulated by glucose.
- IR: decreased insulin sensitivity and/or decreased responsiveness.
- In type 2 Diabetes: defects at multiple levels (receptor and post-receptor).
• HOMA-IR: laboratory testing for insulin resistance
• HOMA-IR: formula → fasting insulin and fasting glucose
• Standardize insulin immunoassay
Thank you