RESISTENSI INSULIN : DEFINISI, MEKANISME PADA DIABETES DAN PEMERIKSAAN LABORATORIUMNYA

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

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 lesion:

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

**Insulin resistance: hallmark of this disorder**
<table>
<thead>
<tr>
<th>Ranking</th>
<th>Country</th>
<th>2000</th>
<th>Country</th>
<th>2030</th>
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<tr>
<td></td>
<td></td>
<td>People with diabetes (millions)</td>
<td></td>
<td>People with diabetes (millions)</td>
</tr>
<tr>
<td>1</td>
<td>India</td>
<td>31.7</td>
<td>India</td>
<td>79.4</td>
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<tr>
<td>2</td>
<td>China</td>
<td>20.8</td>
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<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)

Year

International Diabetes Federation
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 a protein hormone, stored in beta-cells of pancreas as a crystalline form (+Zn)
• Consists of 2 chains, $\alpha$ and $\beta$ chain connected by a disulfide bridge. In alpha chain there is a 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 not be given orally
BIOSYNTHESIS

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

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

- Microsomal enzyme cleave pre-proinsulin 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 granules contain insulin and C-peptide in equimolar amounts.
• Insulin 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. It is metabolized fastly
• Liver is the main organ for insulin metabolism (50%). Other organs are 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.
Insulin secretion stimulation

- Glucose stimulates insulin secretion and suppresses glucagon secretion.

- Initiate by ATP/ADP ratio within the cell → close the membrane ATP-sensitive K channel. → depolarisation of cell → voltage change → opening 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 Insulin secretion

Fig 20.2

Insulin synthesis

plasma insulin

oral glucose

1st phase of insulin secretion

2nd phase

time
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 a member of the growth factor family, glycoprotein membrane is composed of two protein sub units encoded by a single gene.

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

- The alpha sub unit connected by disulfide linkage to the smaller beta subunit MW 95,000 D. This sub unit crosses the membrane and its cytoplasmic domain contains 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 (5 mM)</td>
<td>Basal glucose uptake</td>
</tr>
<tr>
<td>GLUT2</td>
<td>Liver, pancreatic β-cells</td>
<td>High (7-20 mM)</td>
<td>Allows equilibration with blood glucose</td>
</tr>
<tr>
<td>GLUT3</td>
<td>Brain</td>
<td>Low (1 mM)</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 are alpha cells of Langerhans islet ➔ inhibit glucagon secretion.

2. **ENDOCRINE Effect**
   Liver, muscle, adipose tissue
Metabolic effects of insulin

In the liver:

- promotes anabolism
  promotes glycogenesis and glycogen storage
  suppresses lipolysis and promotes synt of protein and lipogenesis

Inhibit gluconeogenesis and promote glycolisis.

- inhibits catabolism
  Inhibiting hepatic glycogenolysis, ketogenesis and gluconeogenesis.
• **In adipose tissue** insulin stimulates triglyceride synthesis and storage, by inducing production of lipoprotein lipase, increases glucose transport, and inhibits intracellular lipolysis

• **In muscle** stimulates protein synthesis, by increasing amino acid transport, promoting glycogenesis
Biological Action of Insulin

Vasodilatation
- ↑ NO release
- ↑ eNOS expression

Platelet inhibition
- ↑ NO release in platelets
- ↑ cAMP

Anti-oxidant
- ↓ ROS generation

Anti-inflammatory
- ↓ NFkB, ↑ IkB
- ↓ MCP
- ↓ ICAM-1
- ↓ CRP

Anti-thrombotic
- ↓ TF

Profibrinolytic
- ↓ PAI-1

Cardio-protective
- Animals, human

Anti-apoptotic
- Heart, other tissues

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

INSULIN RESISTANCE (IR) AND ASSOCIATED CONDITIONS
IR: exists any time, a normal amount of insulin produces a less than normal biologic response. Inadequate tissue response to insulin hormone (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)
- Polycystic ovary disease
- Dyslipidemia
- Decreased fibrinolytic activity
- Acanthosis nigricans
- Hyperuricemia

Adapted from: Omara, A., Beisser, M., & Saad, R. (2010).
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
<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</td>
<td>not important</td>
</tr>
<tr>
<td></td>
<td>or affinity of ins receptor</td>
<td>in DM</td>
</tr>
<tr>
<td>Post receptor</td>
<td>Defect in signal</td>
<td>most probable</td>
</tr>
<tr>
<td></td>
<td>Reduce IRS-1</td>
<td>source of IR</td>
</tr>
<tr>
<td></td>
<td>Decrease pyruvate kinase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>or glycogen synthetase</td>
<td></td>
</tr>
<tr>
<td>GLUT</td>
<td>Defective translocation</td>
<td>Important</td>
</tr>
<tr>
<td></td>
<td>of GLUT to cell membrane</td>
<td></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

Figure 9.17. A unifying hypothesis for the pathogenesis of type 2 diabetes and insulin resistance based on results from various tissue-specific knockout mice.
Insulin Resistance in:
1. Muscle: increased accumulation of fat and secondary insulin resistance, hypertriglyceridemia, and increased levels of free fatty acids.
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. Diejomaoh 3
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>
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<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>
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<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>
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<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>
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<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>
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<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>
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<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>
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<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>
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<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>
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</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

Pierre Allard,² Edgard E. Delvin,² Gilles Paradis,⁴ James A. Hanley,⁴ Jennifer O’Loughlin,⁴ Claudette Lavallée,⁵ Emile Levy,³ and Marie Lambert¹*

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

Allison B. Goldfine,¹* Robert W. Gerwien,² Janice A. Kolberg,² Sheila O’Shea,¹ Sarah Hamren,² Glenn P. Hein,² Xiaomei M. Xu,² and Mary Elizabeth Patti¹
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</th>
<th>Estimate</th>
<th>Standard error</th>
<th>$P$</th>
<th>Multiple $R^2$</th>
<th>Bootstrap $R^2$</th>
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<tr>
<td>Insulin sensitivity (all markers)</td>
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<td></td>
<td></td>
<td>0.91</td>
<td>0.90</td>
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<tr>
<td>Glucose</td>
<td>-0.646</td>
<td>0.2231</td>
<td>0.0048</td>
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<tr>
<td>Insulin</td>
<td>-0.852</td>
<td>0.0413</td>
<td>&lt;0.0001</td>
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<td>Fas ligand</td>
<td>-0.011</td>
<td>0.0051</td>
<td>0.0303</td>
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<td>Complement C3</td>
<td>-0.0001</td>
<td>0.00004</td>
<td>0.0073</td>
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<tr>
<td>PAI-1</td>
<td>-0.122</td>
<td>0.0535</td>
<td>0.0248</td>
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<tr>
<td>Insulin sensitivity (excluding glycemic markers and insulin)</td>
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<td></td>
<td>0.62</td>
<td>0.58</td>
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<tr>
<td>IGFBP-1</td>
<td>0.239</td>
<td>0.0453</td>
<td>&lt;0.0001</td>
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<td>Leptin</td>
<td>-0.171</td>
<td>0.0359</td>
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<td>PAI-1</td>
<td>-0.220</td>
<td>0.0813</td>
<td>0.0083</td>
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<td>GPT</td>
<td>-0.291</td>
<td>0.0856</td>
<td>0.0010</td>
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<tr>
<td>Triglycerides</td>
<td>-0.223</td>
<td>0.0801</td>
<td>0.0067</td>
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</tbody>
</table>
Insulin Testing

Method: 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, K$_3$-EDTA, and sodium citrate plasma, avoid hemolysis
Sample stability: room temperature 8 h; 2-8°C 24 h; $\leq$ -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,\textsuperscript{1*} Linda M. Thienpont,\textsuperscript{2} Katleen Van Uytfanghe,\textsuperscript{2} Penelope M. Clark,\textsuperscript{3} Patrik Lindstedt,\textsuperscript{4} Göran Nilsson,\textsuperscript{5} and Michael W. Steffes,\textsuperscript{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,\textsuperscript{1} Katleen Van Uytfanghe,\textsuperscript{1} Veronique Stove,\textsuperscript{2} Tom Fiers,\textsuperscript{2} and Linda M. Thienpont\textsuperscript{1*}
<table>
<thead>
<tr>
<th>Error component</th>
<th>Notation</th>
<th>Possible sources</th>
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<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>
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<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>
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<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>
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<tr>
<td></td>
<td></td>
<td>6. Error in the assigned value of the reference material used for preparation of calibrators</td>
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<td>7. Variation between vials of reference material</td>
</tr>
<tr>
<td></td>
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<td>8. Errors in the preparation of the calibrators</td>
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<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>
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<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.
CONCLUSION

- Insulin resistance: hallmark of type 2 Diabetes mellitus
- 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 level (receptor and post receptor)
• HOMA-IR : laboratory testing for insulin resistance
• HOMA-IR : formula → fasting insulin and fasting glucose
• Standardize insulin immunoassay
Thank you