Carbohydrate metabolism

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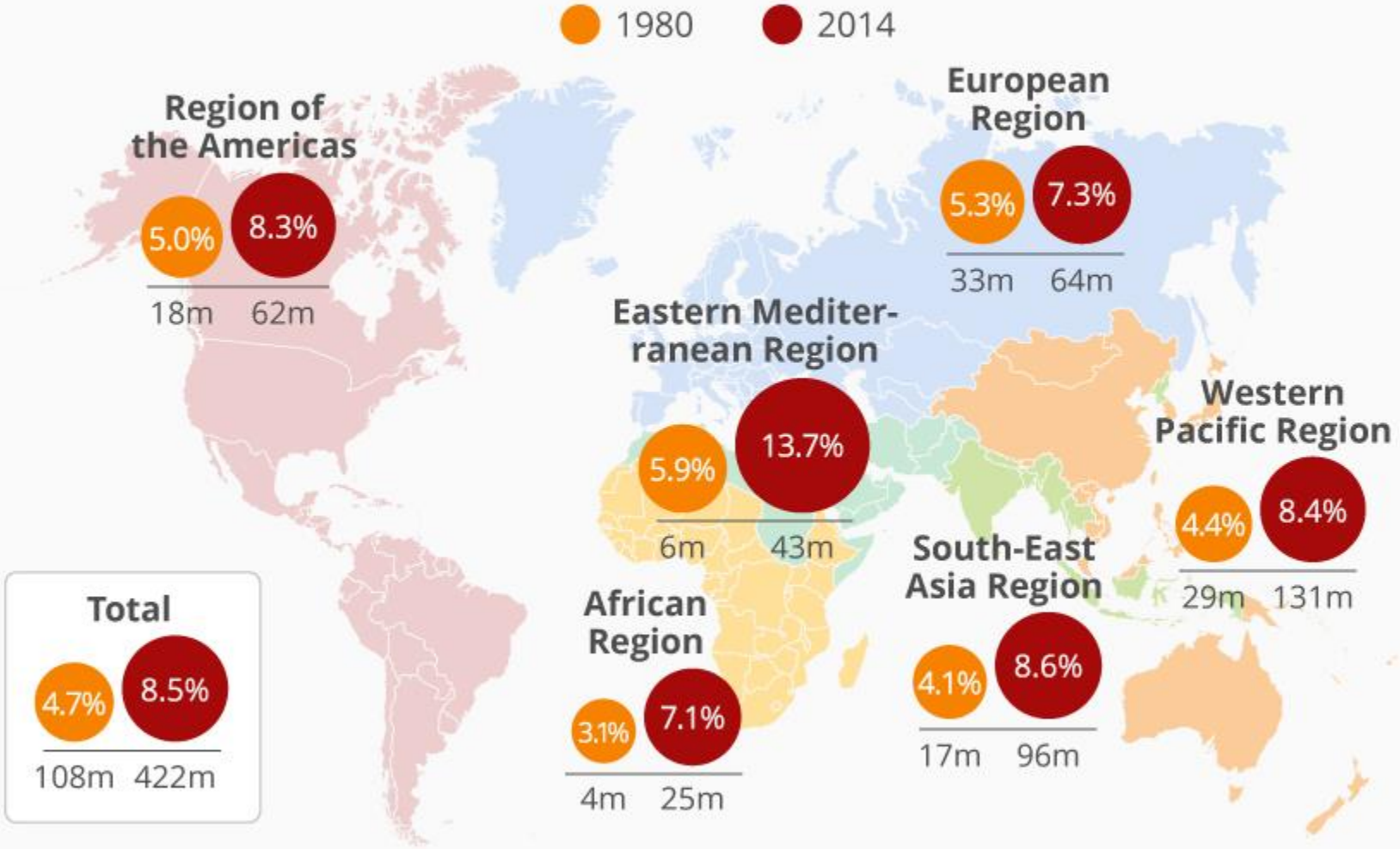
The Unrelenting March Of Diabetes

% prevalence and number of adults with diabetes by WHO region in 1980 and 2014*

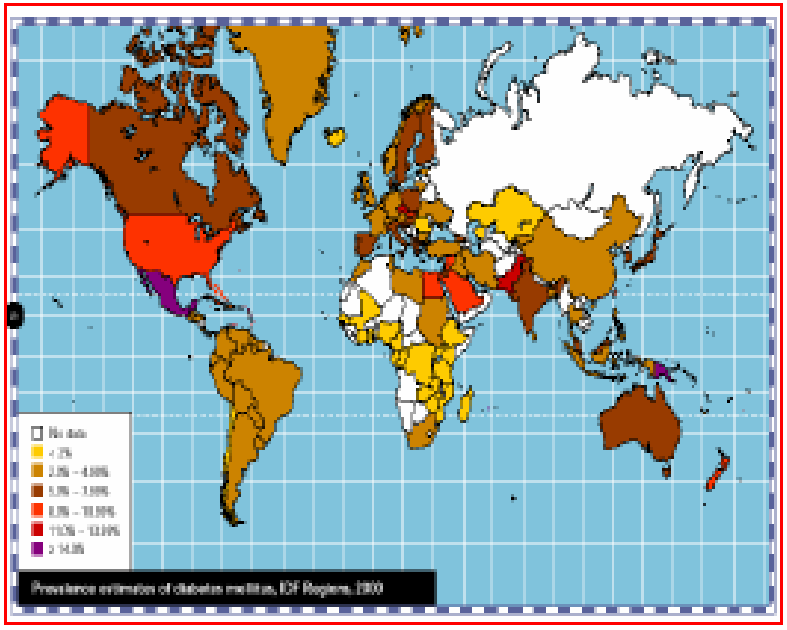


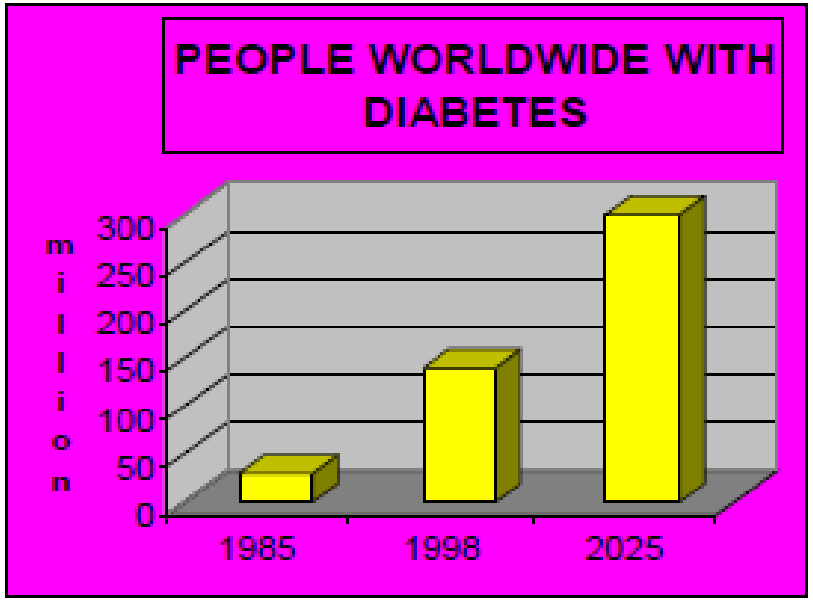
* Millions of people and % of total regional population

Source: World Health Organization

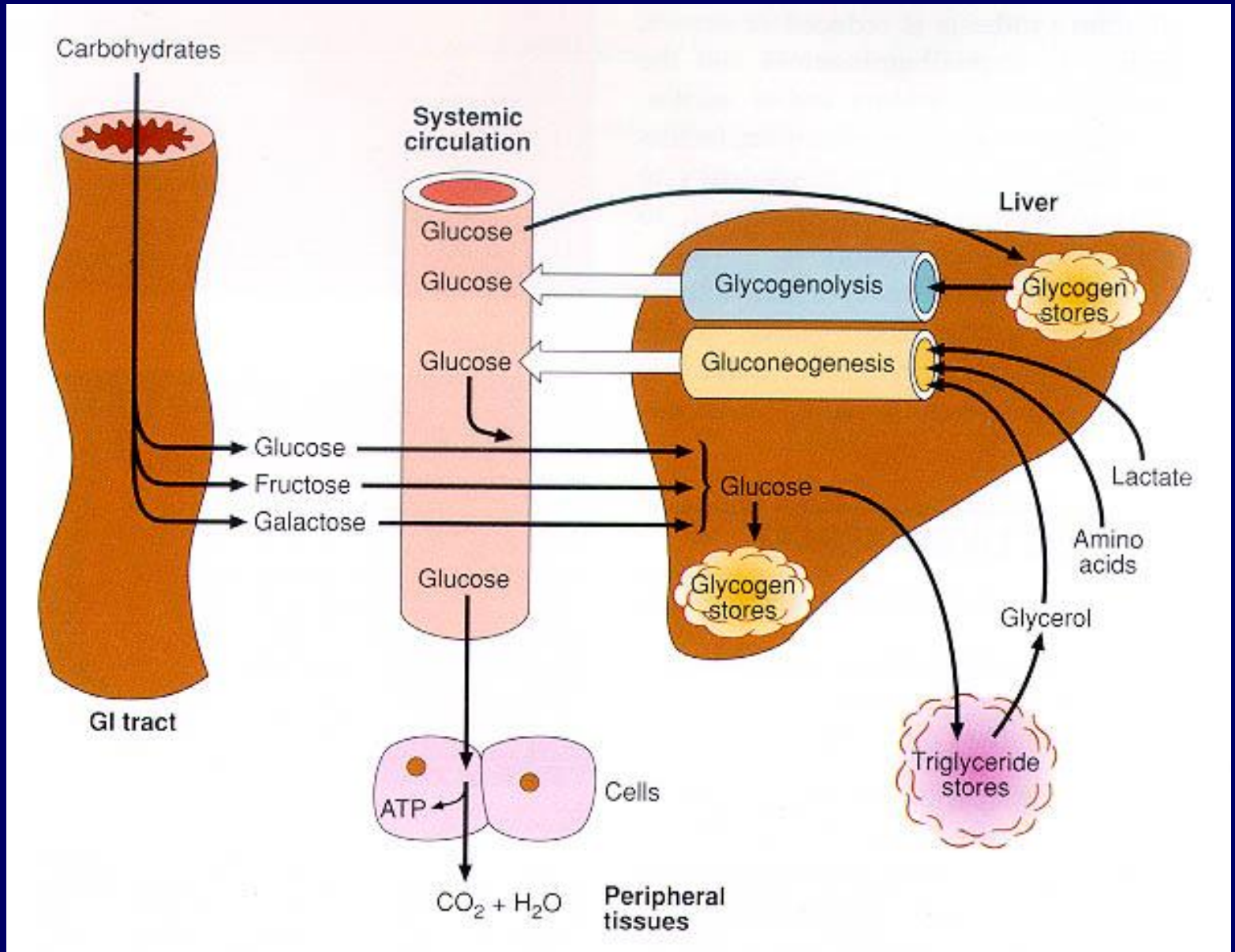
THE PREVALENCE OF DIABETES MELLITUS

- A syndrome caused by a decrease or total lack of insulin or diminished effectiveness of circulating insulin (insulin resistance)
- Characterized by hyperglycemia
- „Westernized lifestyle"
- The biggest healthcare challenge of the 21st century.
- 8 % annual increase

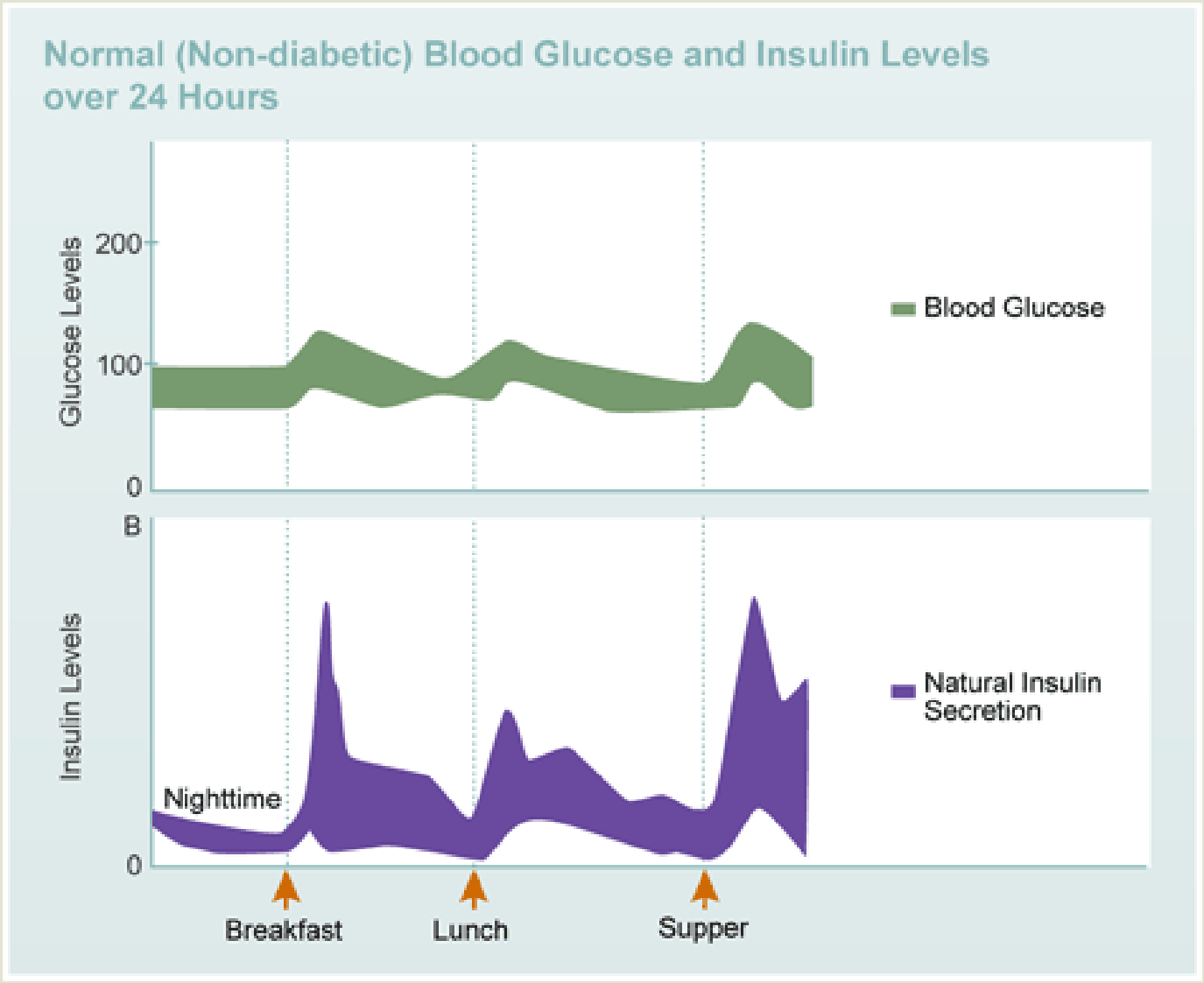




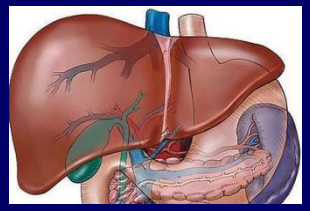
Carbohydrate metabolism



Insulin secretion in view of nutrition

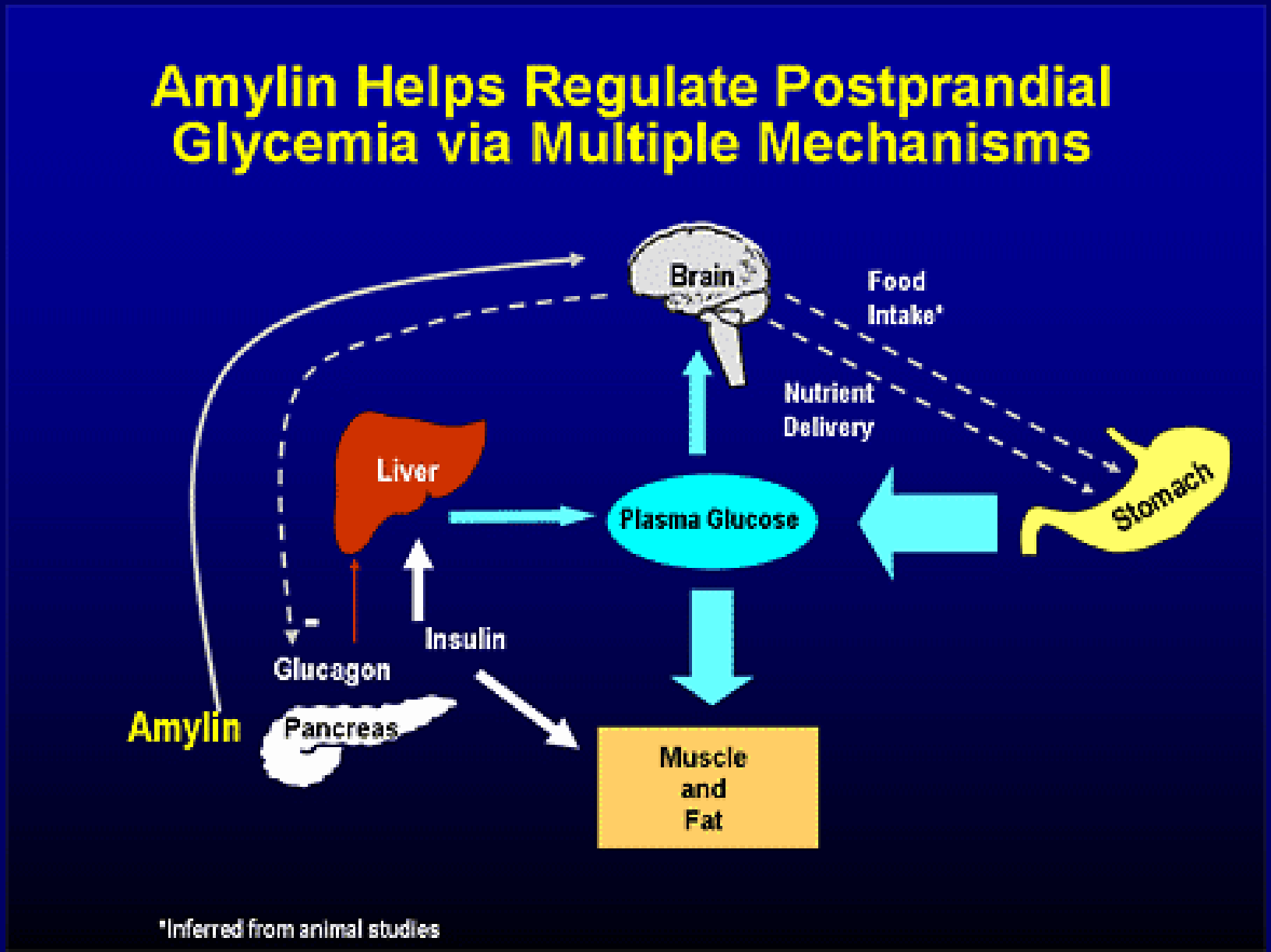


Homeostasis

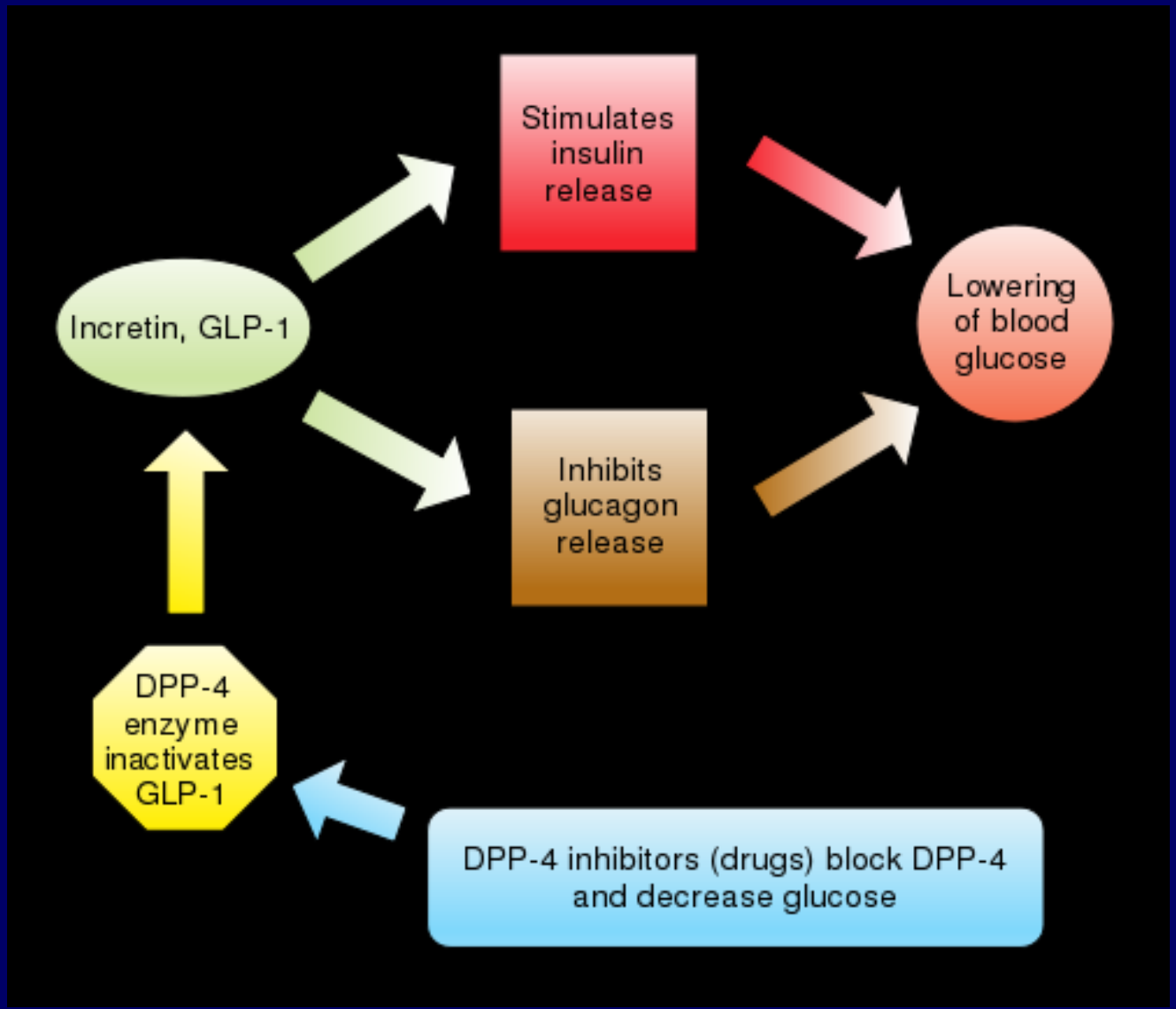


Hormon	Effect	Tissue	Glucose
Insulin	Cellular glucose uptake ⇑ Glycogen- and protein synthesis ⇑ Fatty acids- and triglyceride synthesis ⇑ Gluconeogenesis, glycogenolysis ⇓ Ketogenesis, lipolysis, proteolysis ⇓ **Growth factor at the same time!**	Muscle, fat issue Muscle, liver Fat issue Muscle, liver Muscle, fat issue, liver	⇓
Glucagon	Gluconeogenesis, glycogenolysis ⇑ Ketogenesis, lipolysis ⇑	Liver Muscle, liver	⇑
Amylin	Appetite ⇓ Gastric emptying ⇓	Brain Stomach	⇓
Adrenalin	Glycogenolysis ⇑ Lipolysis ⇑	Muscle, liver Fat issue	⇑
GH	Glycogenolysis ⇑ Lipolysis ⇑	Liver Fat issue	⇑
Cortisol	Gluconeogenesis, glycogen synthesis ⇑ Proteolysis ⇑ Tissue glucose utilization ⇓	Liver Muscle Muscle, fat issue, liver	⇑
GLP-1	Insulin synthesis ⇑ Gastric emptying ⇓	Pancreas ß cells Stomach	⇓

Insulin+Amylin



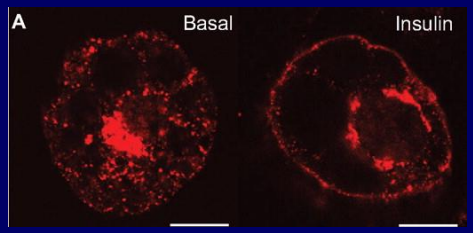
Glucagon like peptid (GLP1)

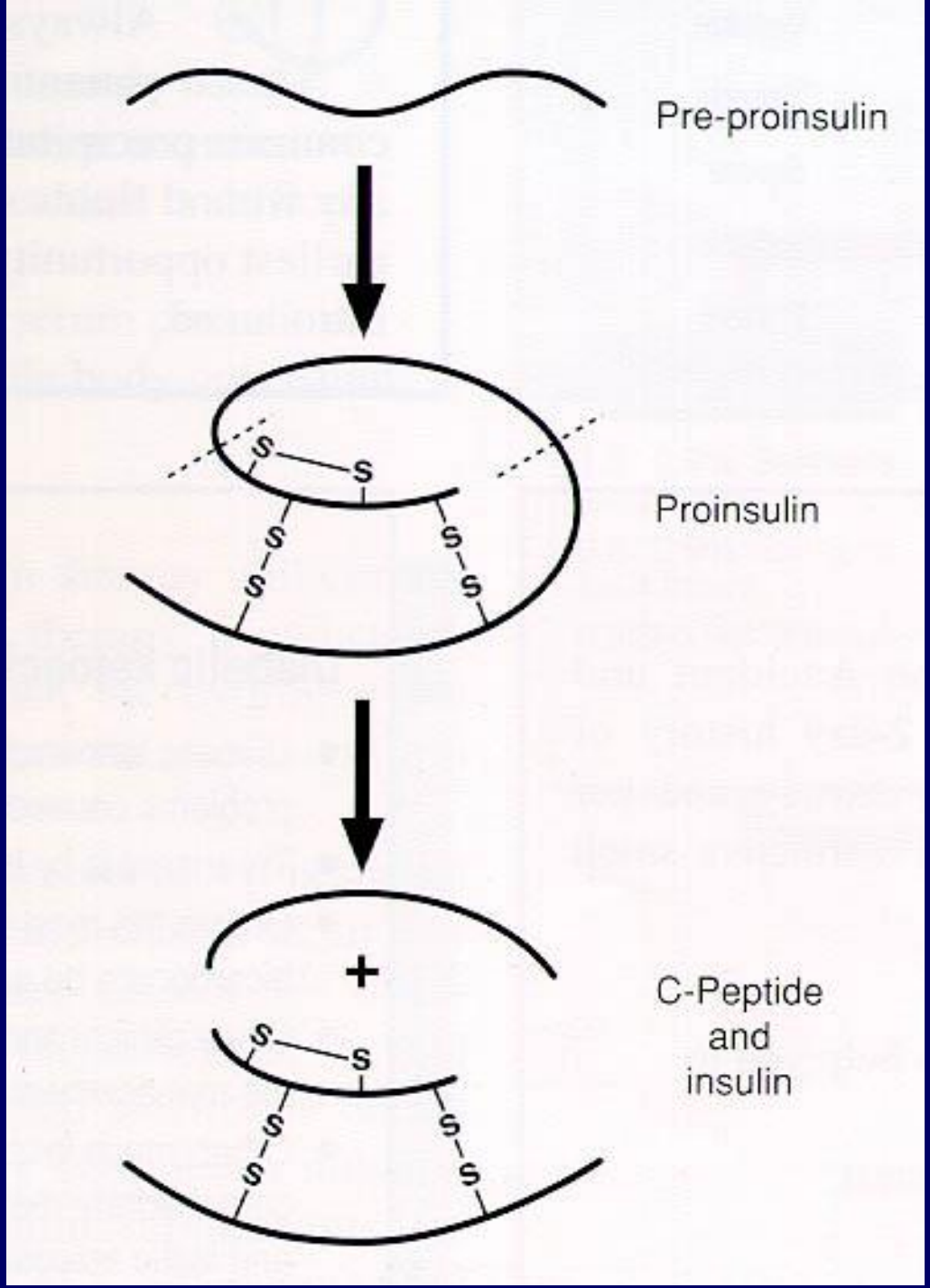


GLUT (glucose transporters)

- **GLUT1**: Embryonal cells, erythrocytes, endothelial cells (Decreased synthesis if plasma glucose is high)
- **GLUT2**: Kidneys, liver, pancreas ß cells
- **GLUT3**: Neurons, placenta
- **GLUT4: Fat tissue, muscle (cardiac, skeletal)**

  Insulin sensitive
- **GLUT5**: Fructose transporter





The birth of insulin

Endogenous insulin: C-peptide!

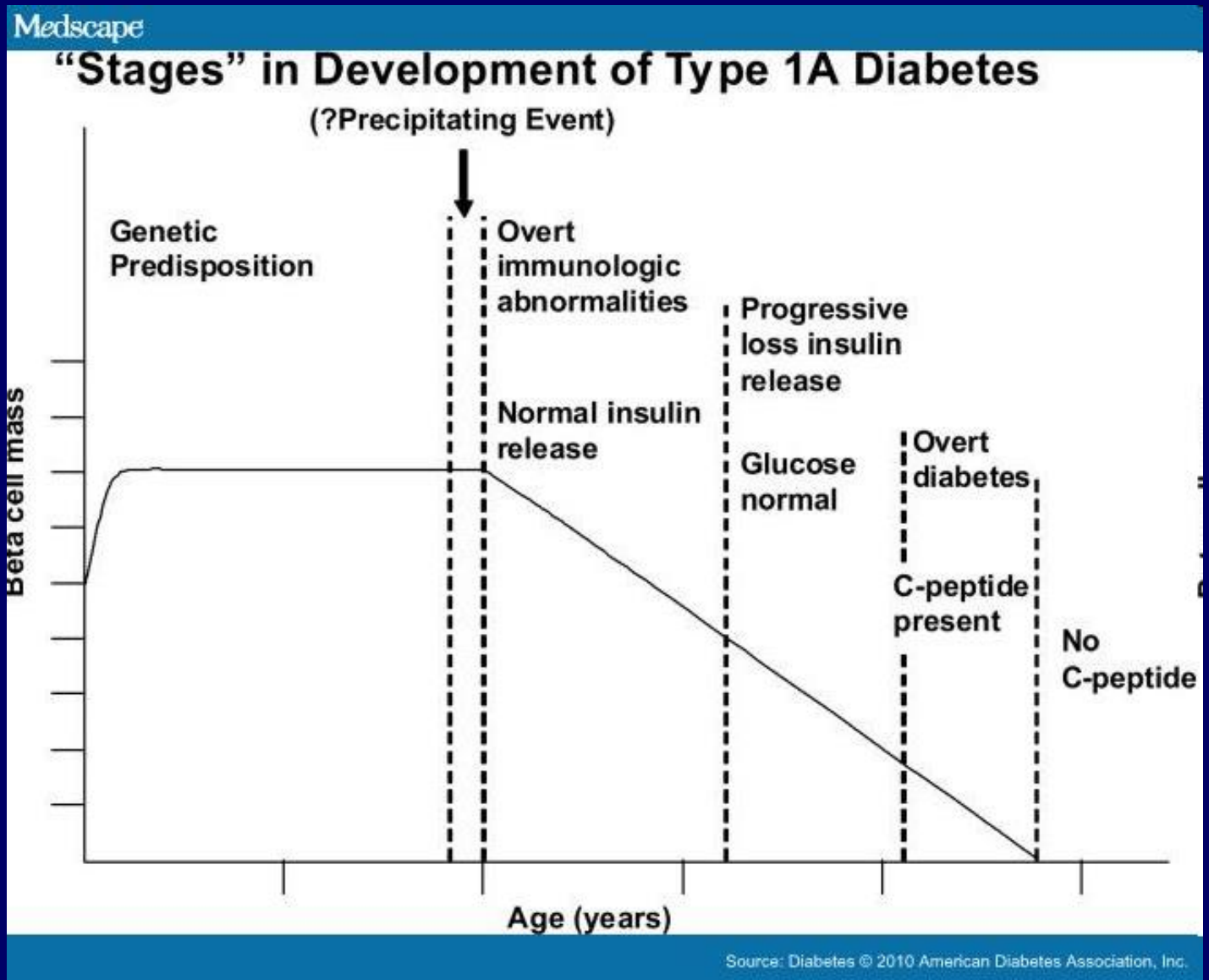
Diabetes mellitus

- **Type 1**
- B-cell destruction
- Insulin deficiency
- Keto-acidosis
- Rapid onset
- Autoimmune + virus
- At young age
- Genetical predisposition
- 1 : 100

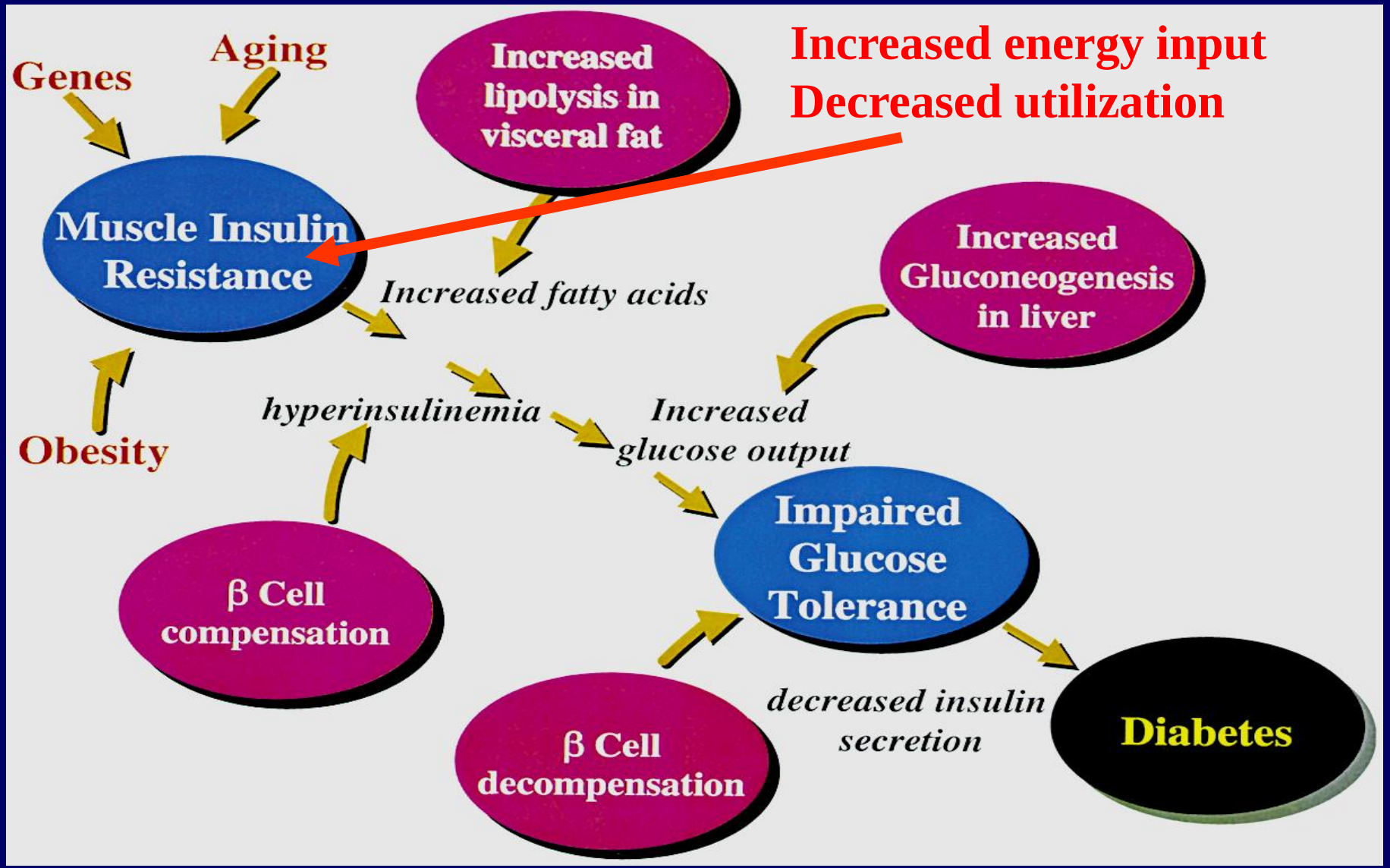
- **Type 2**
- Insulin resistance
- Insulin maybe high
- Keto-acidosis is rare
- Slow onset
- Lifesyte-dependent
- Obese adults
- Genetical predisposition
- 1 : 10-20

Insulin determination from the 60ies

Development of diabetes mellitus type 1



Development of diabetes mellitus type 2



Importance of preanalytical factors in testing carbohydrate metabolism

- **Sample types: capillary, venous plasma, venous whole blood**
- **Glycolysis inhibitor (NaF or iodoacetamide)**
- **Anticoagulant (heparin)**
- **Timing of sample collection (in the morning, postprandial, glucose tolerance test)**
- **Patient preparation (diet, fasting blood sample)**

Reference range is sample type dependent!

Importance of laboratory tests in diabetes mellitus

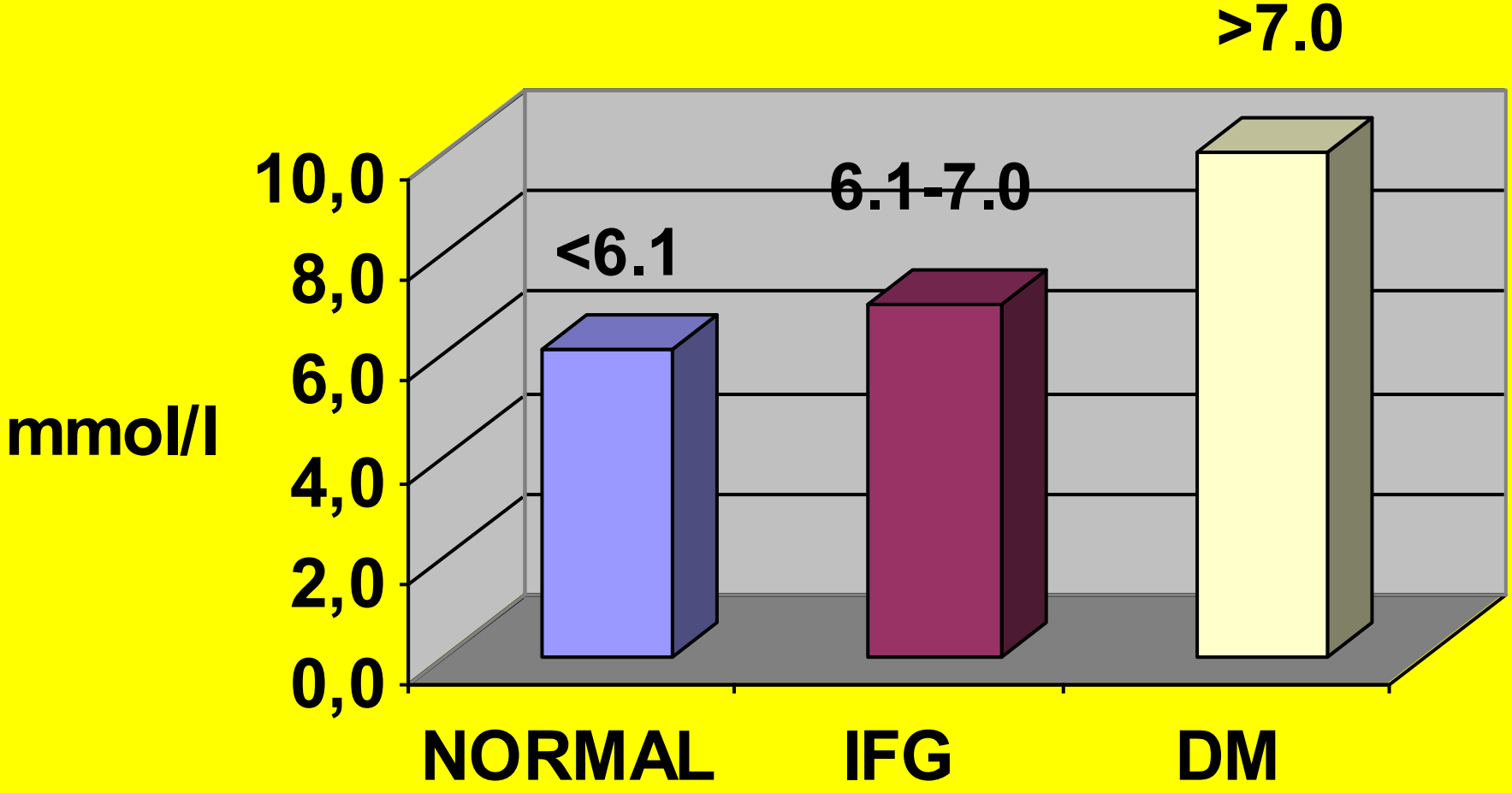
- Diagnostic importance!
- Negative predictive value!
- Life saving importance!
- Utmost importance in monitoring
- Enables monitoring at home
- Suitable for long term assessment
- Suitable for detection of early complications

Laboratory diagnostics of diabetes mellitus

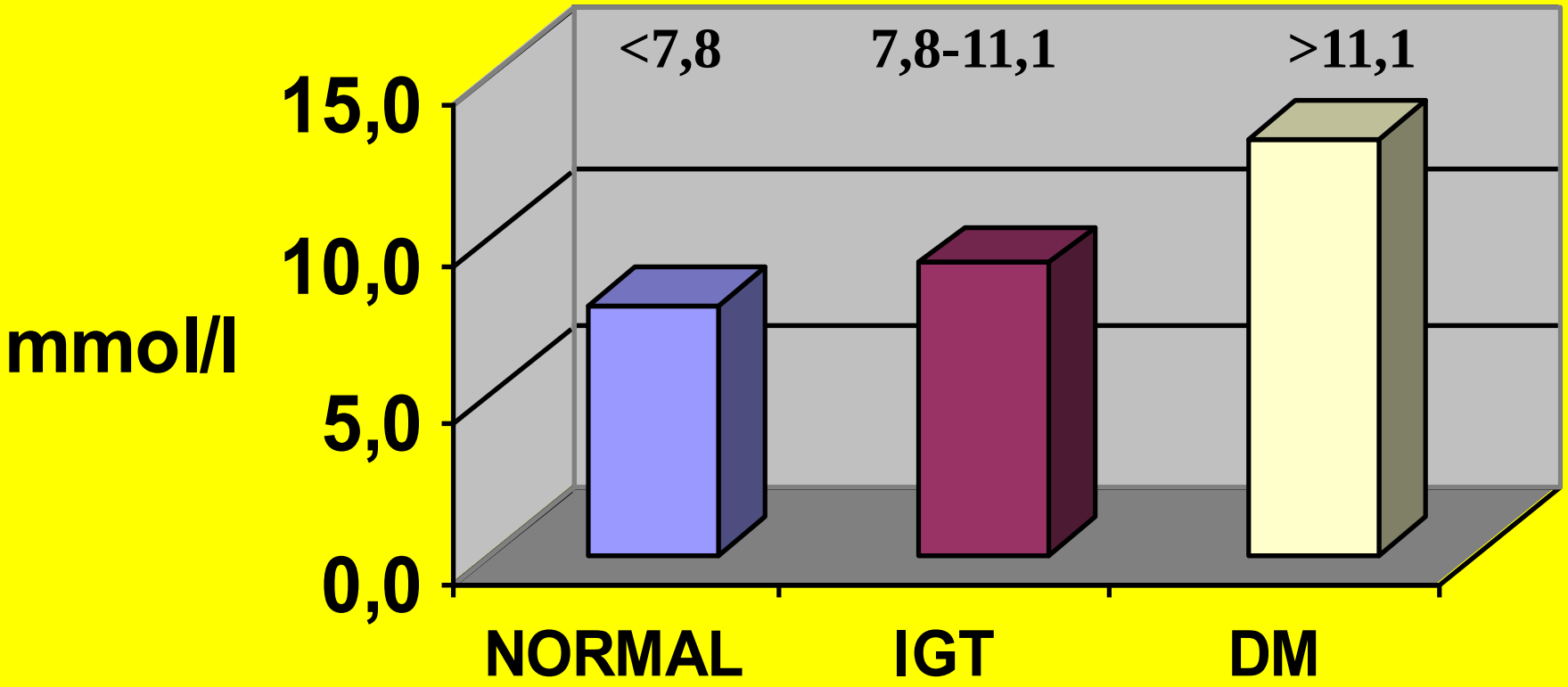
- **Fasting plasma glucose!**
- **Postprandial (random) plasma glucose**
- **Oral glucose tolerance test**
- **Glucose tolerance test with insulin profile**
- **Kidney function**

- **Urinalysis: total protein, microalbumin, general parameters (ketones)**

FASTING PLASMA GLUCOSE



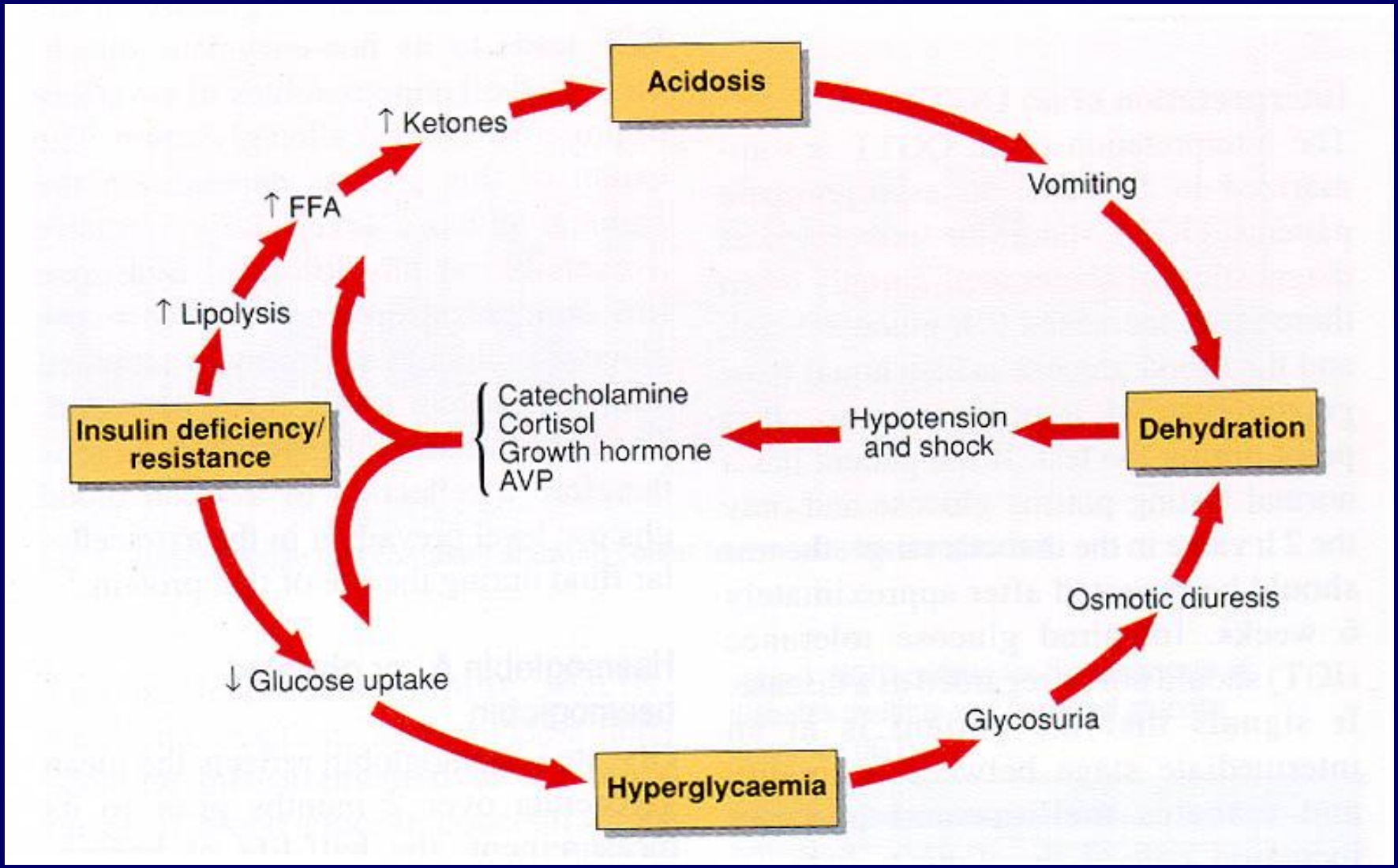
ORAL GLUCOSE TOLERANCE (2 h)



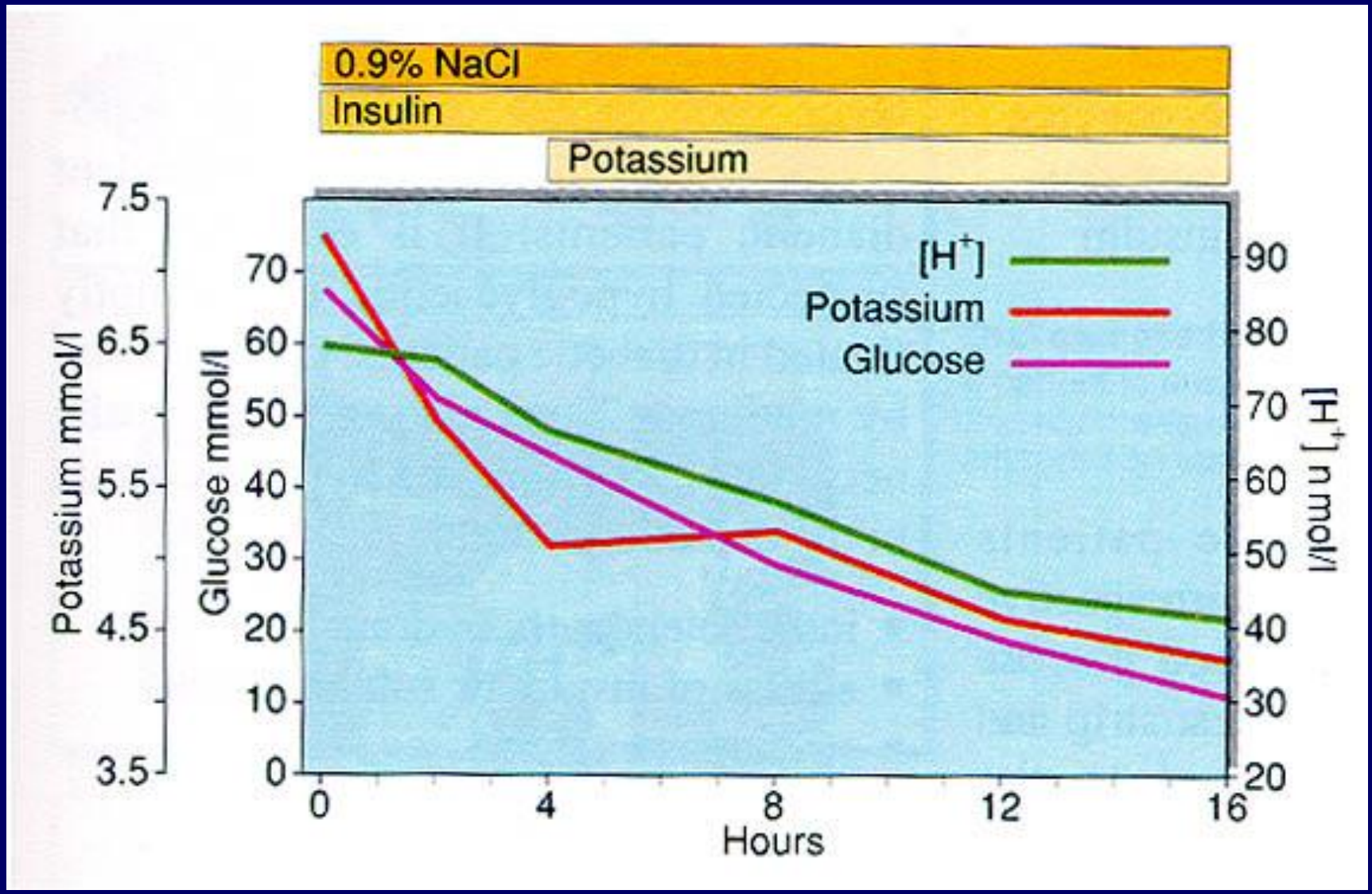
Acute complications of diabetes mellitus

Features	Diabetic keto-acidosis (DKA)	Hyperosmolar non–ketotic coma (HONK)	Lactic acidosis
Plasma glucose	High	Very high	Variable
Ketones	Present	None	Variable
Acidosis	Moderate/Severe	None	Severe
Dehydration	Prominent	Prominent	Variable
Hyperventilation	Present	None	Present

Diabetic ketoacidosis



Treatment and monitoring of ketoacidosis



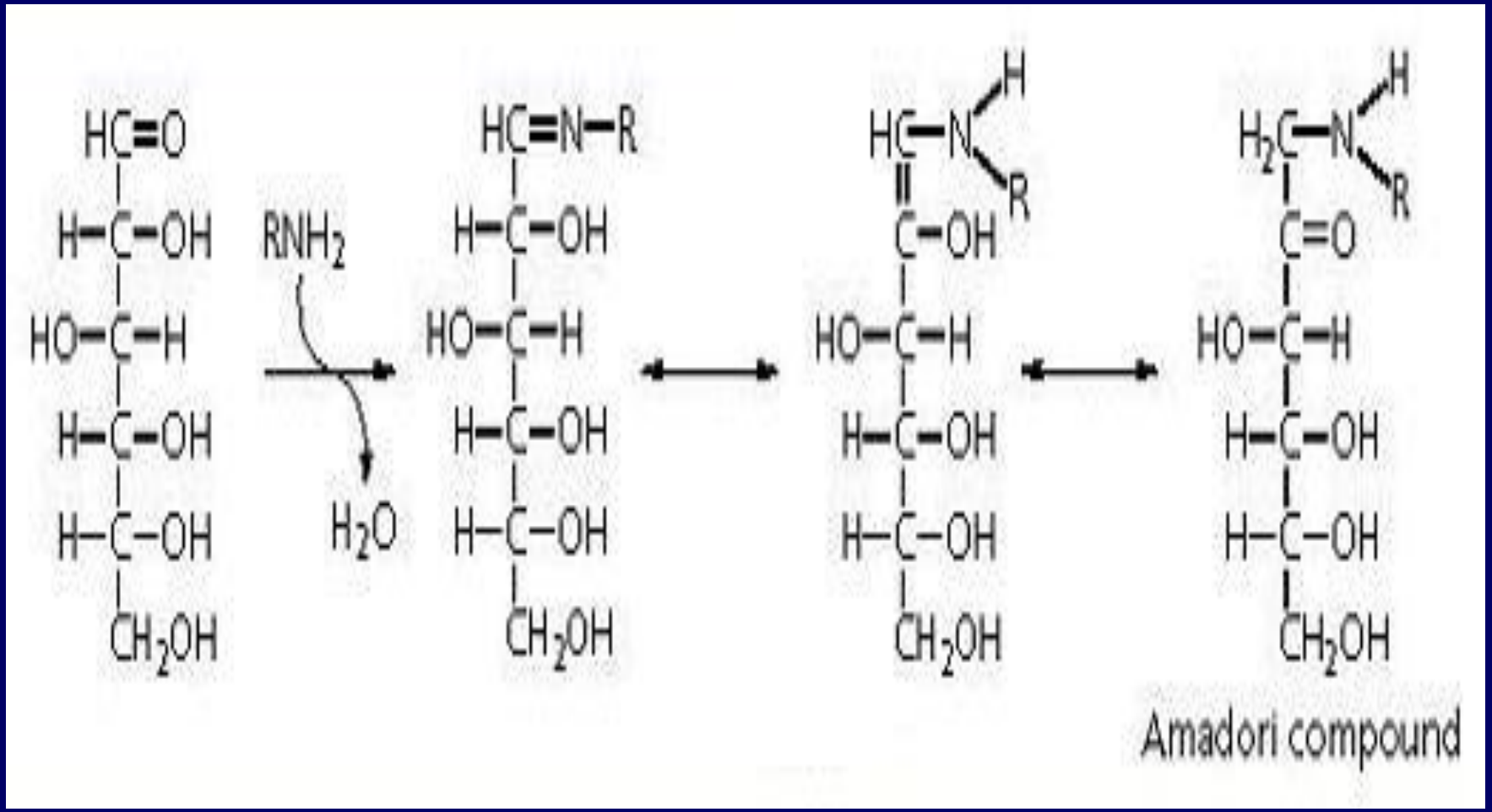
Laboratory monitoring of diabetes mellitus

- Fasting plasma glucose
- Glucose tolerance test (limited indications!)
- Fructosamine (glycated albumin, in every 2-3 weeks)
- HbA1c (in every 3 months)
- Kidney function, electrolytes (K, Ca), water balance, microalbuminuria
- Lipid parameters
- Insulin, C-peptide

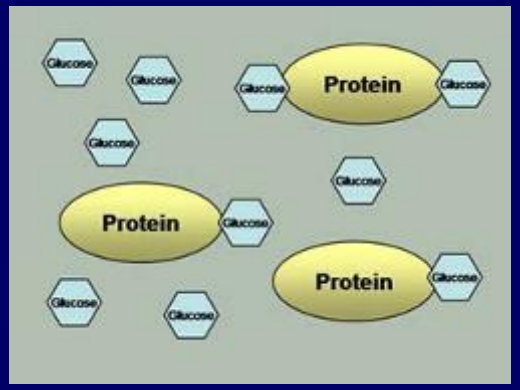
Diabetes mellitus- monitoring

- Blood glucose POCT – at home
- from capillary blood, immediate determination by a semi-quantitative device ⇒ Insulin dosage!

Non-enzymatic glycation of proteins: Amadori reaction



Fructosamine

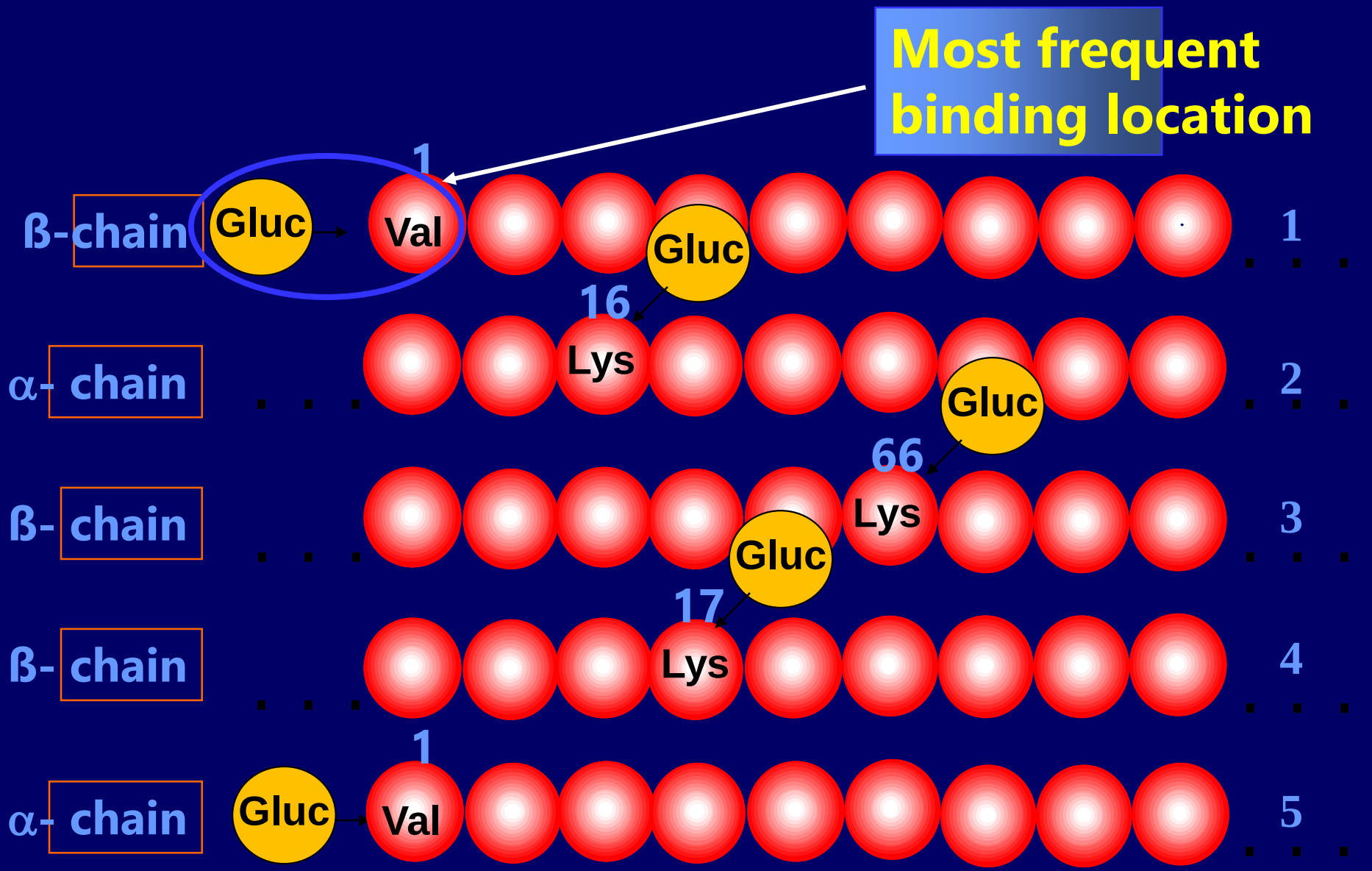


- Glycated plasma proteins
- Albumin
- 200-285 μmol/l
- 2-3 weeks biological half-life
- Disturbed protein functions: e.g. nephropathy

Glycated hemoglobin

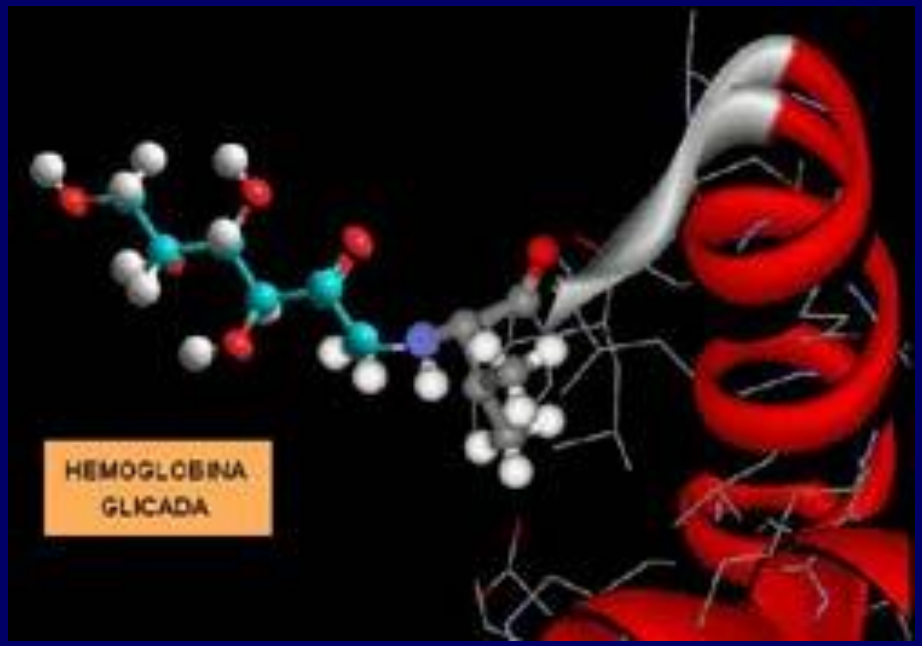
- Hemoglobin forms:
  - HbA: 2 α and 2 β chain 95-97%
    - HbA_0 : non-glycated 90%
    - HbA_1 : glycated hemoglobin
      - HbA_{1a1} Fructose-1,6-diphosphate
      - HbA_{1a2} Glucose-6-phosphate
      - HbA_{1b} other sugars
      - **HbA_{1c} 75-80% HbA_1 : β chain N-terminal valine gycated with D-glucose**
  - HbA_2 2 α and 2 δ chain <3%
  - HbF 2 α és 2 γ chain <1% Fetal hgb

Long-term monitoring: glycated hemoglobin



Significance of HbA1c

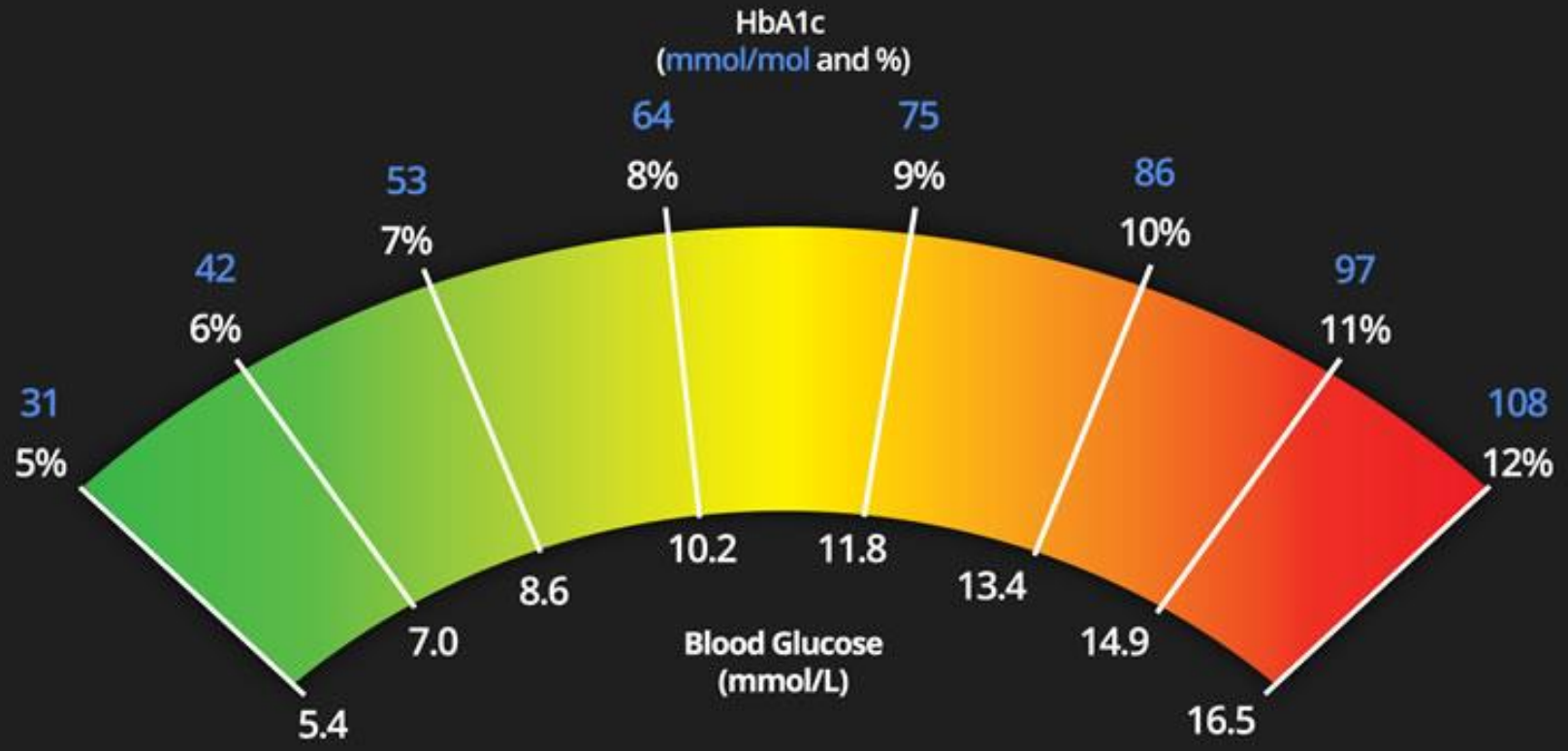
- RBC lifetime: 100-120 days
  - Concentration of HBA1c mirrors the mean glucose level of the previous 3 months if hemoglobin synthesis/degradation is normal



* monitoring: 3 monthly
  - %
  - **mmol HbA1c / mol Hb**

POCT HbA1c

HbA1c as an indicator of Diabetes Control

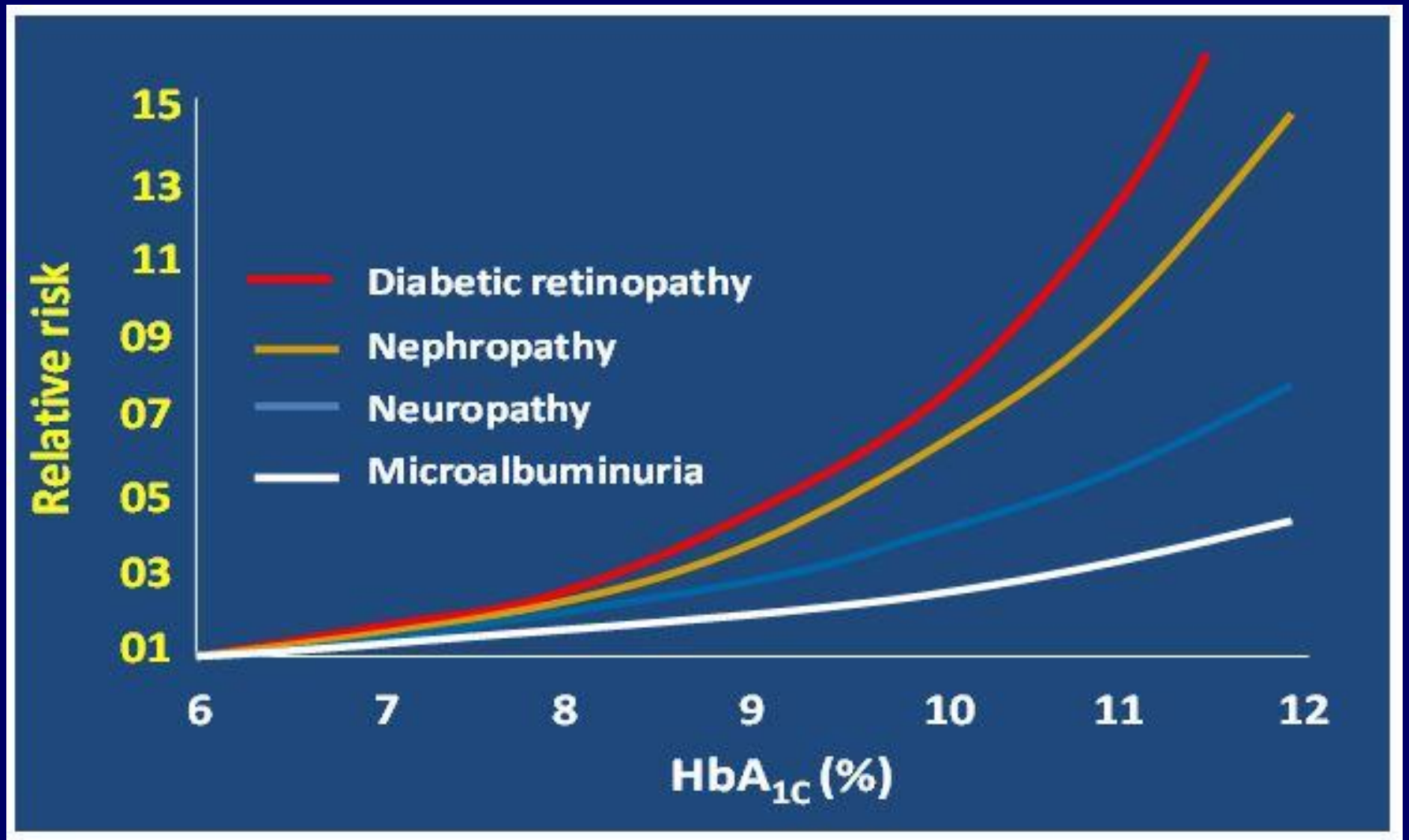


Diabetes.co.uk
the global diabetes community

unite for diabetes

Nathan et al. Translating the A1C Assay into Estimated Average Glucose Values. Diabetes Care 2008; 31:1473-1478
Christchurch Diabetes Centre 2009

Risk of complications in diabetes mellitus vs. HbA1c



Consequences of non-enzymatic glycation in general

1. Schiff base (non-enzymatic reaction of glucose with lysine residues of protein) →
2. Irriversible Amadori product →
3. Advanced glycation end product(AGE)

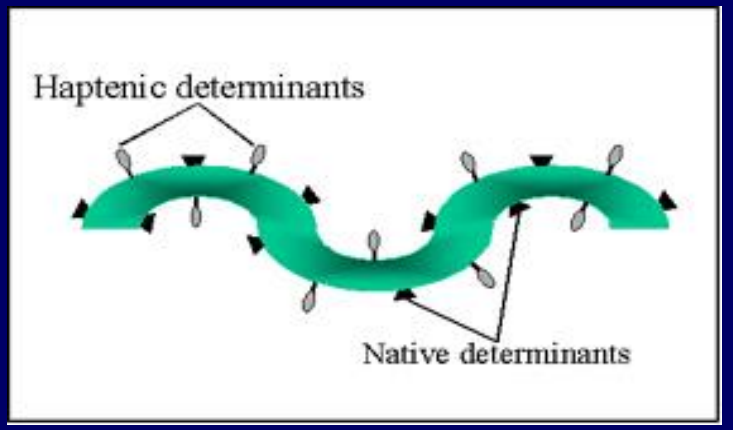
Alteration of intracellular protein function
Interference with ECM function
Increased cytokine and free radical formation through interaction with AGE receptors
May lead to oxidative stress and activation of NKκB.

Principles of immunological methods

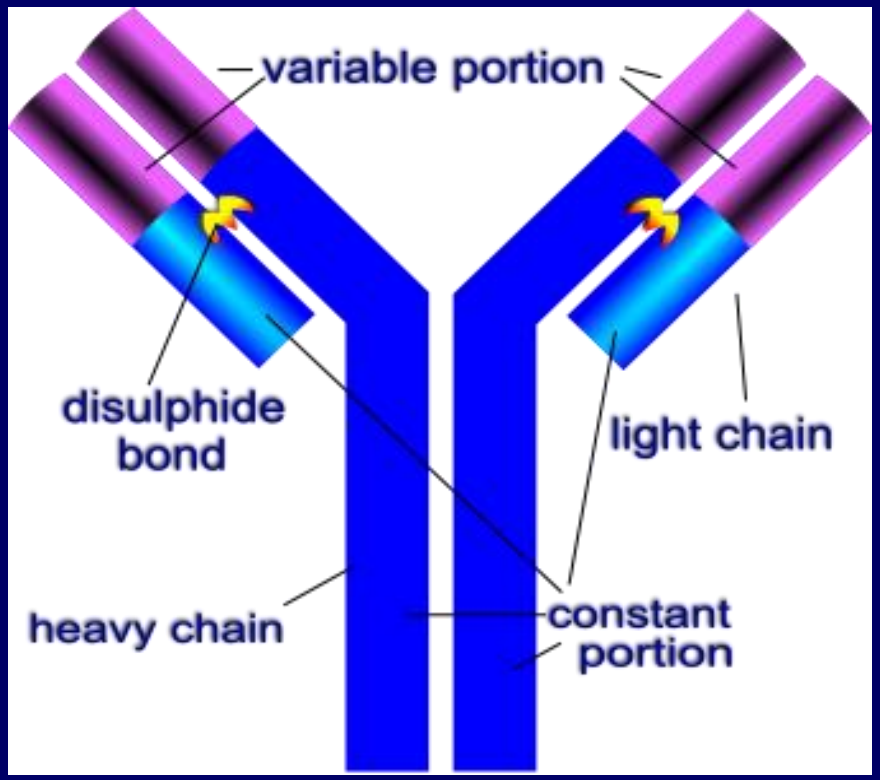
- **Antibody: monovalent - polyvalent**
  polyclonal - monoclonal
- **Immune reaction: solid phase (heterogenous)**
  homogenous
  saturation type
  competitive
- **By labels: RIA, EIA, FIA, FPIA, LIA,**

Basics - antigen

- **Complete – induces immune response (proteins, polysaccharides, nucleic acids)**
- **Hapten – small molecule, not immunogenic, only together with a carrier protein**



Basics - antibody



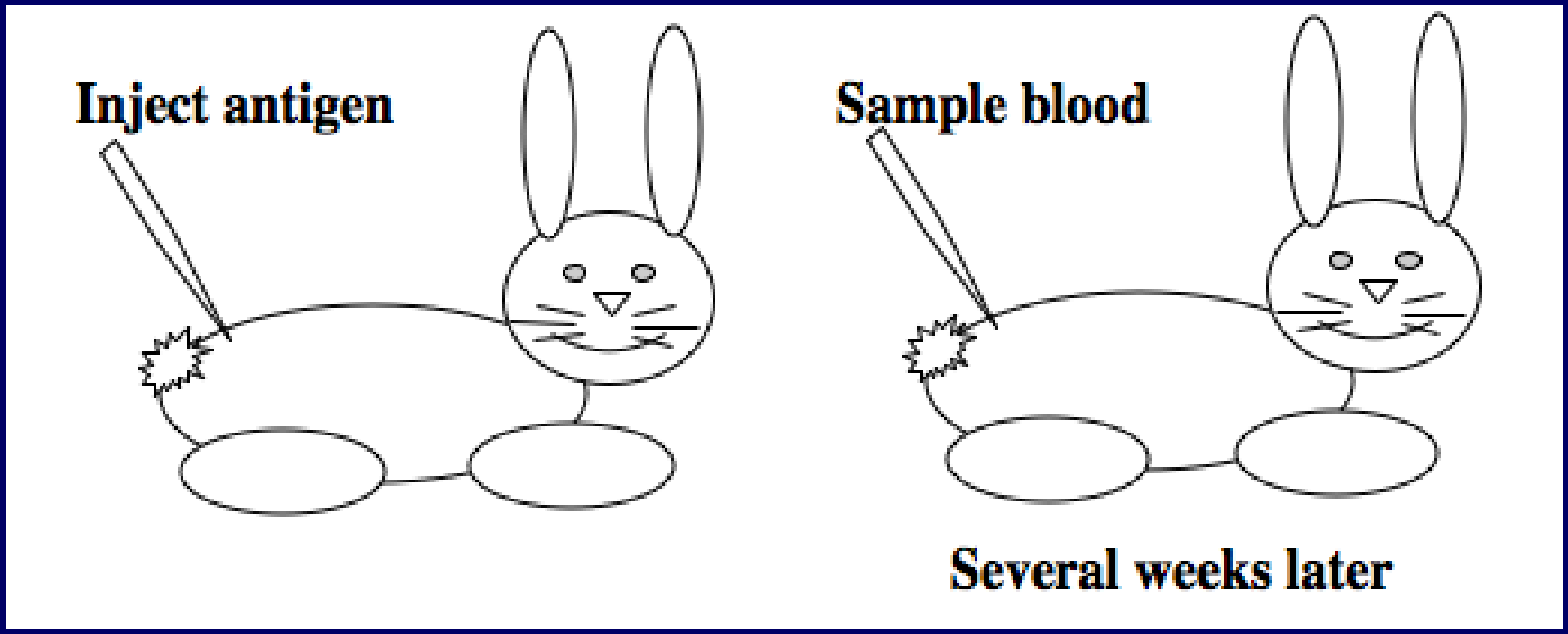
Antigen binding site

Light chain

Heavy chain

Basics - antibody

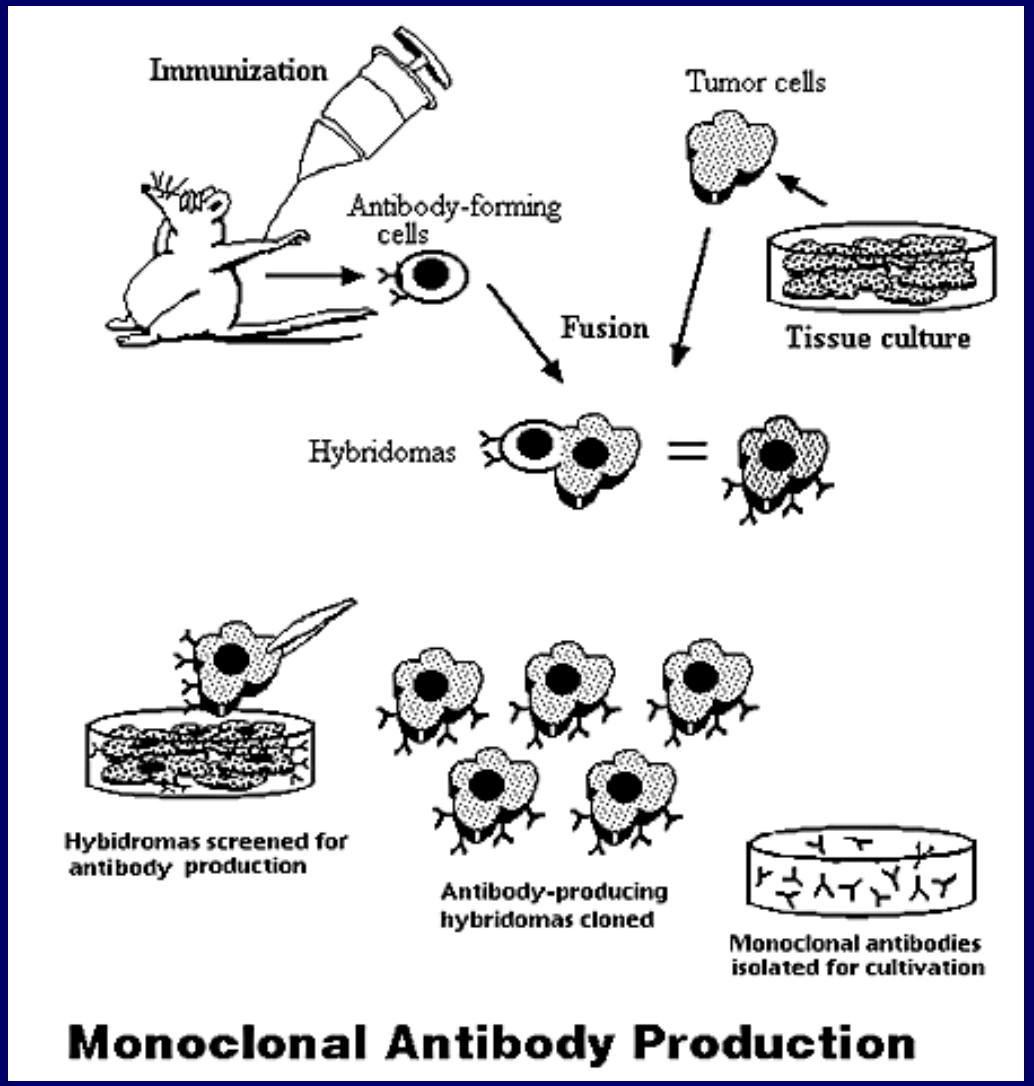
- **Polyclonal, monovalent antibody**



Basics - antibody

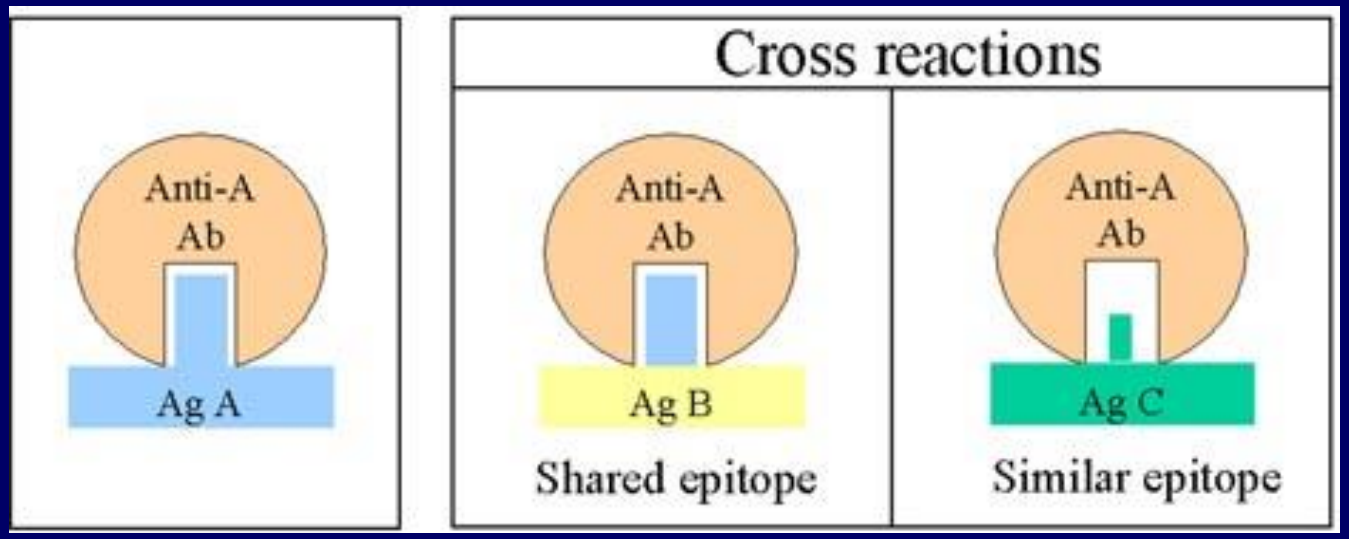
- **Monoclonal, monovalent antibody**

Niels K. Jerne, Georges J.F. Köhler és César Milstein, 1984



Antigen – antibody reaction

- **Specificity: recognition of one epitope (monoclonal antibodies) or recognition of several epitopes on a single molecule (polyclonal antibodies)**
- **Cross reactions:**



Definition of an immunoassay

- Antigen – antibody reaction based sensitive and specific method which is suitable for quantitative determination of very low concentration of antigenic molecules.

- During the measuring process labels are used to detect the reaction and the method is named after the applied label (RIA 1959, FIA, EIA, LIA, ECLIA, FPIA, stb.)

The beginning

RIA method in 1959-1960

Rosalyn Yalow and Soloman Berson

Solely manual methods

Basics of immunoassays

- **Solid phase – heterogenous assays**

  separation of bound/free antigen is required (washing steps)

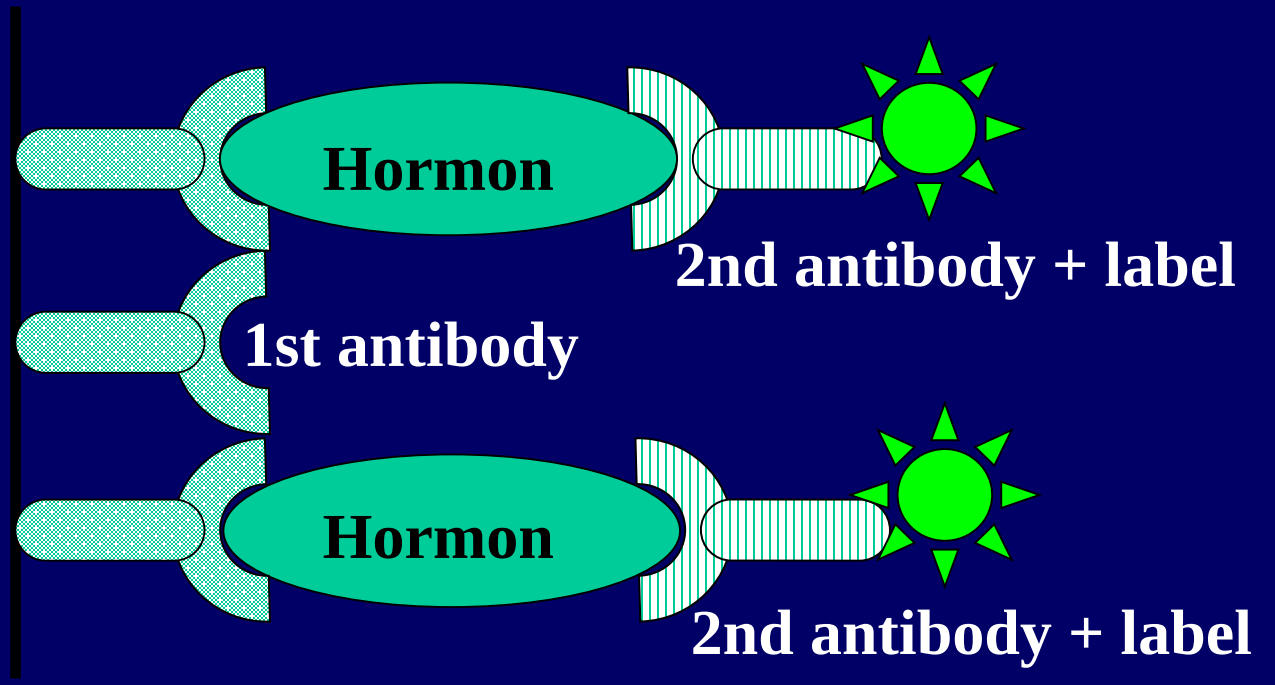
- **Homogenous assays**

  separation is not required

- **Both assays: saturation type or competitive**

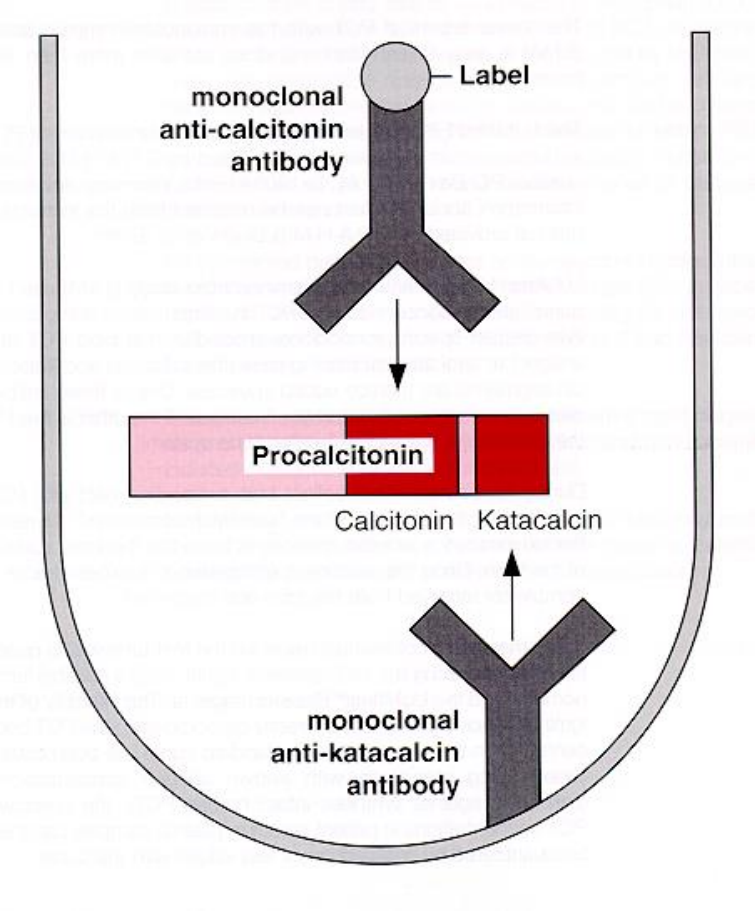
Heterogenous saturating assay

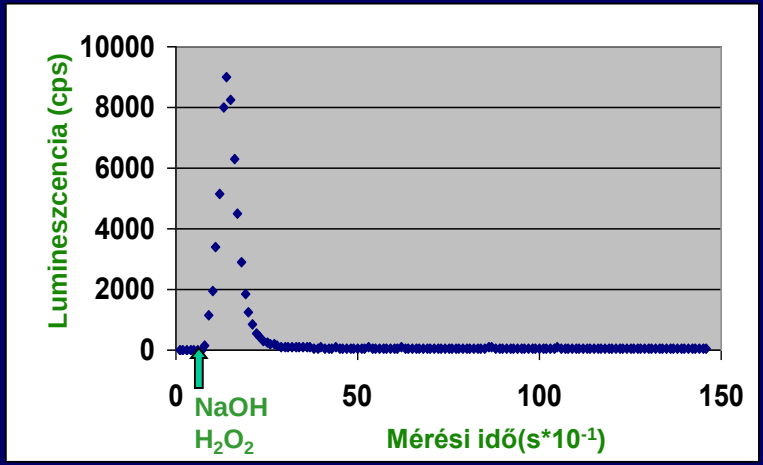
Hormone measurement with solid phase immunoassay (sandwich)



RIA
FIA
LIA
EIA

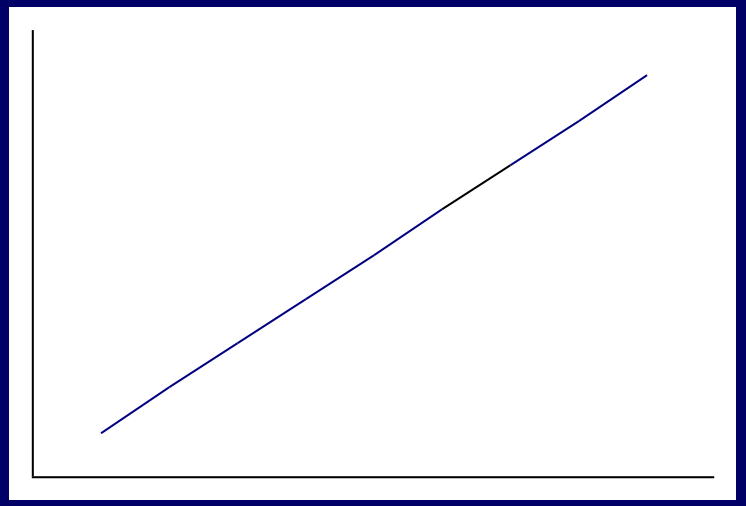
Acridinium ester labeled chemiluminescence immunoassay

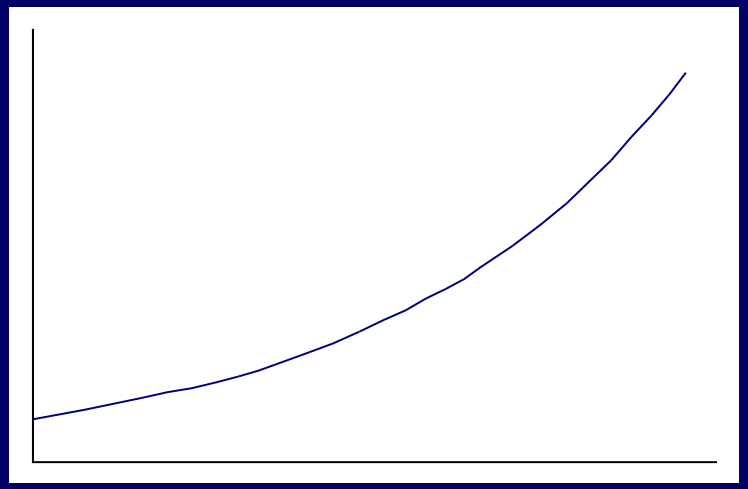




Solid phase sandwich immunoassay

Calibration curve of a saturating immunoassay



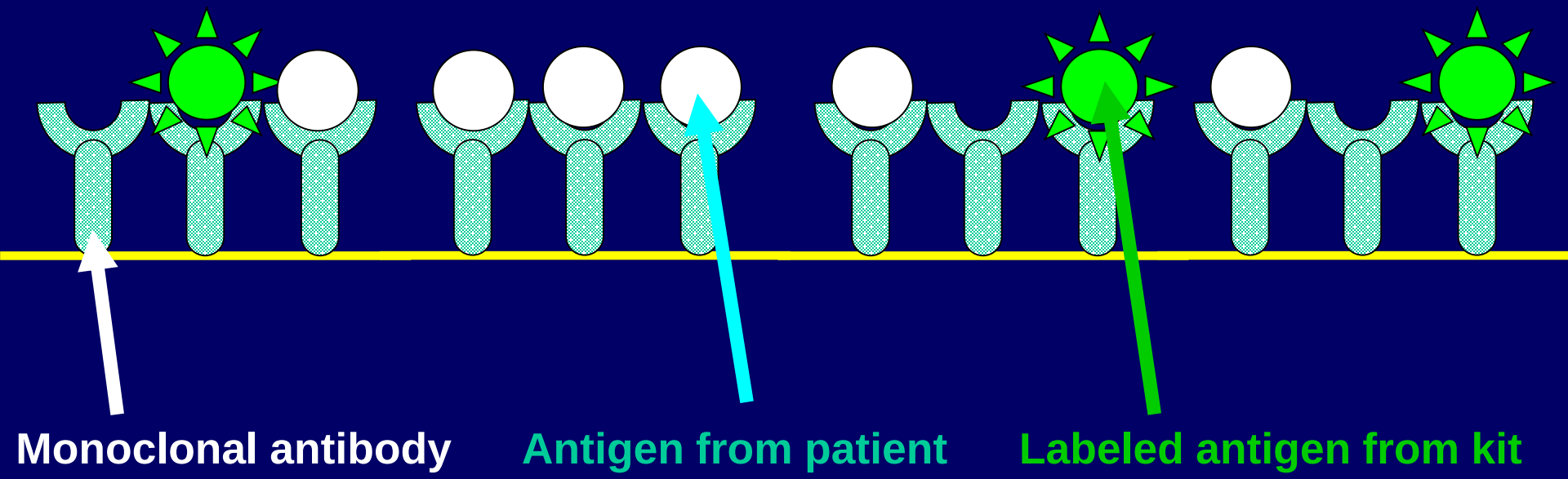


Calibration:

6 points

or 2 points (master)

Competitive heterogenous immunoassay

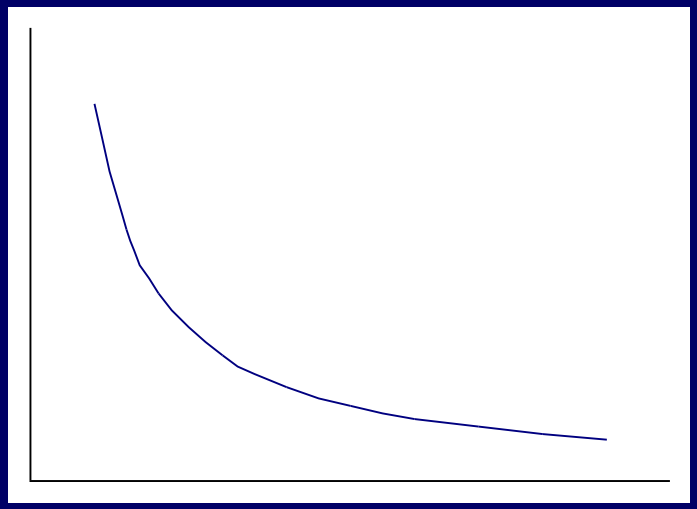


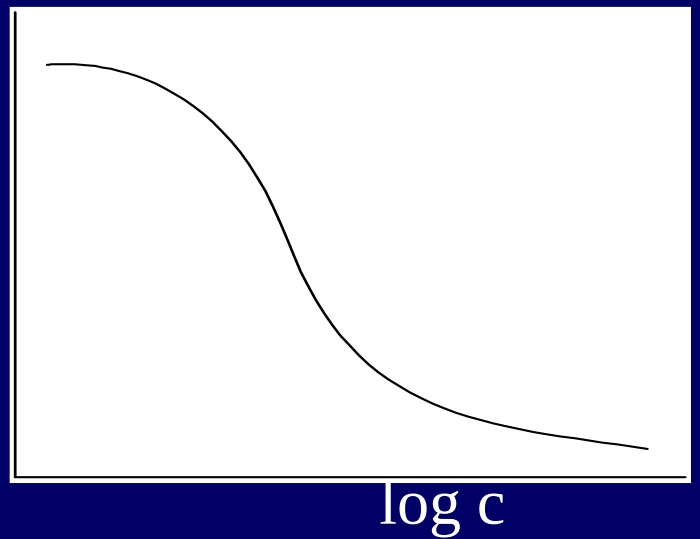
Monoclonal antibody

Antigen from patient

Labeled antigen from kit

Calibration curve of competitive immunoassay





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