Lecture Notes on Chemical Pathology and Diagnosis of DM
(Role of Clinical Laboratory Medicine)

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Diabetes: Definition

Group of metabolic diseases

Insulin secretion → **Hyperglycemia** ← **Insulin Action**

Disturbances of carbohydrate, fat and protein metabolism

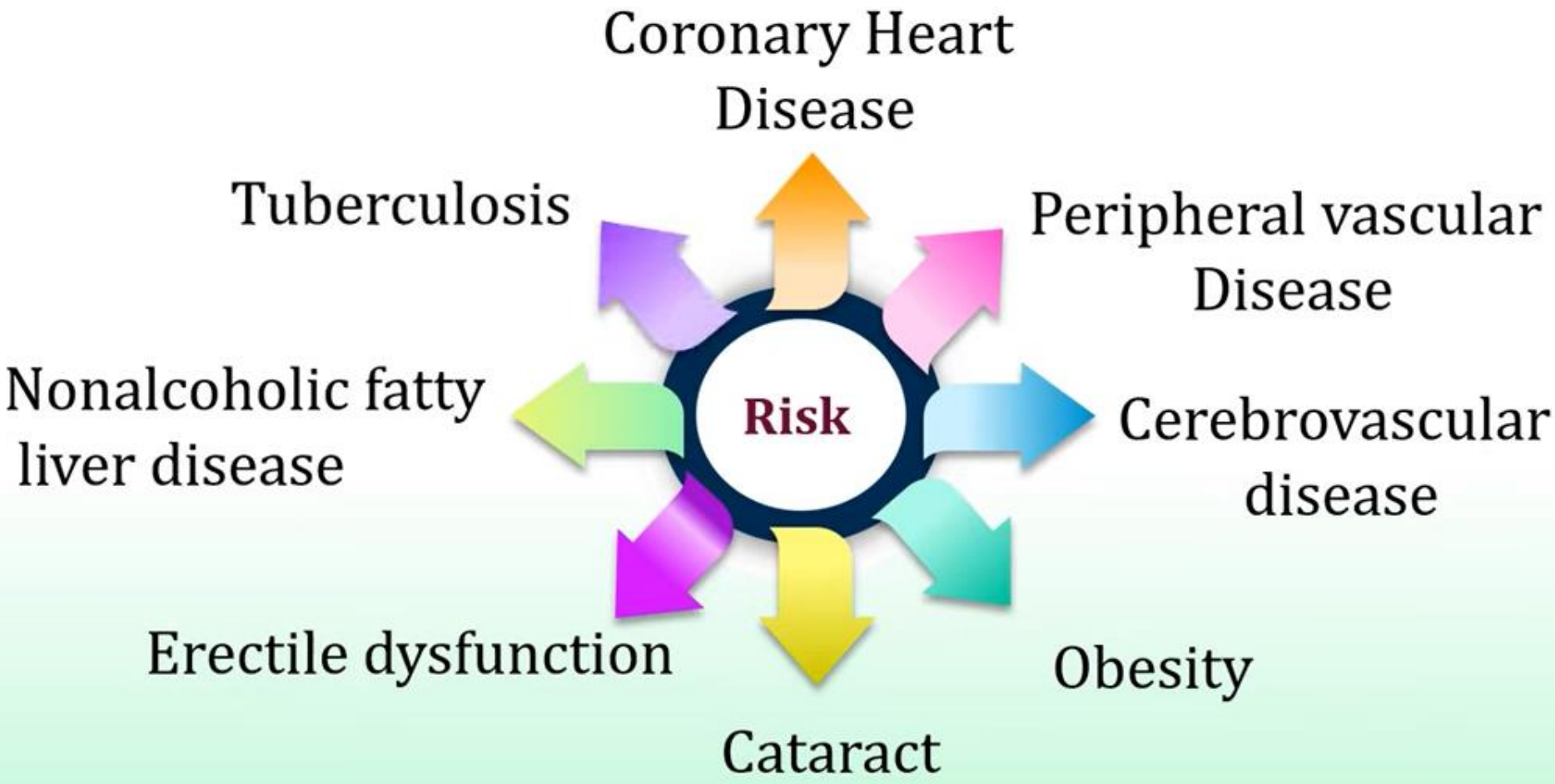
Long-term damage, dysfunction, and failure of different organs

Eyes | **kidneys** | **Heart** | **Blood vessels** | **Nerves**





Diabetes: Increased risk



Classification of Diabetes

1 **Type 1**

- Autoimmune disease
- Pancreatic β-cell destruction and
- **Absolute deficiency of insulin.**

2 **Type 2**

- Peripheral resistance to insulin action **(Insulin resistance)**
- Inadequate secretory response by the pancreatic β cells

("Relative insulin deficiency")

3 **Specific types of diabetes due to other causes**

- Monogenic diabetes syndromes (Neonatal diabetes , MODY),
- Diseases of the exocrine pancreas (cystic fibrosis, pancreatitis),

4

Diabetes: Diagnostic Criteria: ADA guidelines

FPG ≥ 126 mg/dL (7.0 mmol/L)
Fasting is defined as no caloric intake for at least 8 h.*

OR

2-h PG ≥ 200 mg/dL (11.1 mmol/L) during OGTT
(WHO guidelines: using a glucose load 75 g anhydrous glucose dissolved in water.)*

OR

HBA_1C ≥ 6.5% (48 mmol/mol)
(Method NGSP certified and standardized to the DCCT assay.)*

OR

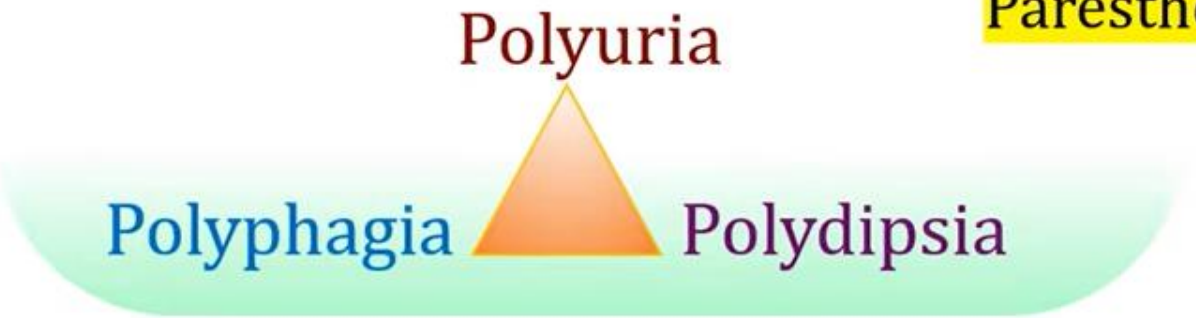
A random plasma glucose ≥ 200 mg/dL (11.1 mmol/L)
(patient with classic symptoms of hyperglycemia or hyperglycemic crisis)

*In the absence of unequivocal hyperglycemia, diagnosis requires two abnormal test results from the same sample or in two separate test samples

Type 1 Diabetes

- Autoimmune disease
- Pancreatic β-cell destruction and
- **Absolute deficiency of insulin**

Paresthesia



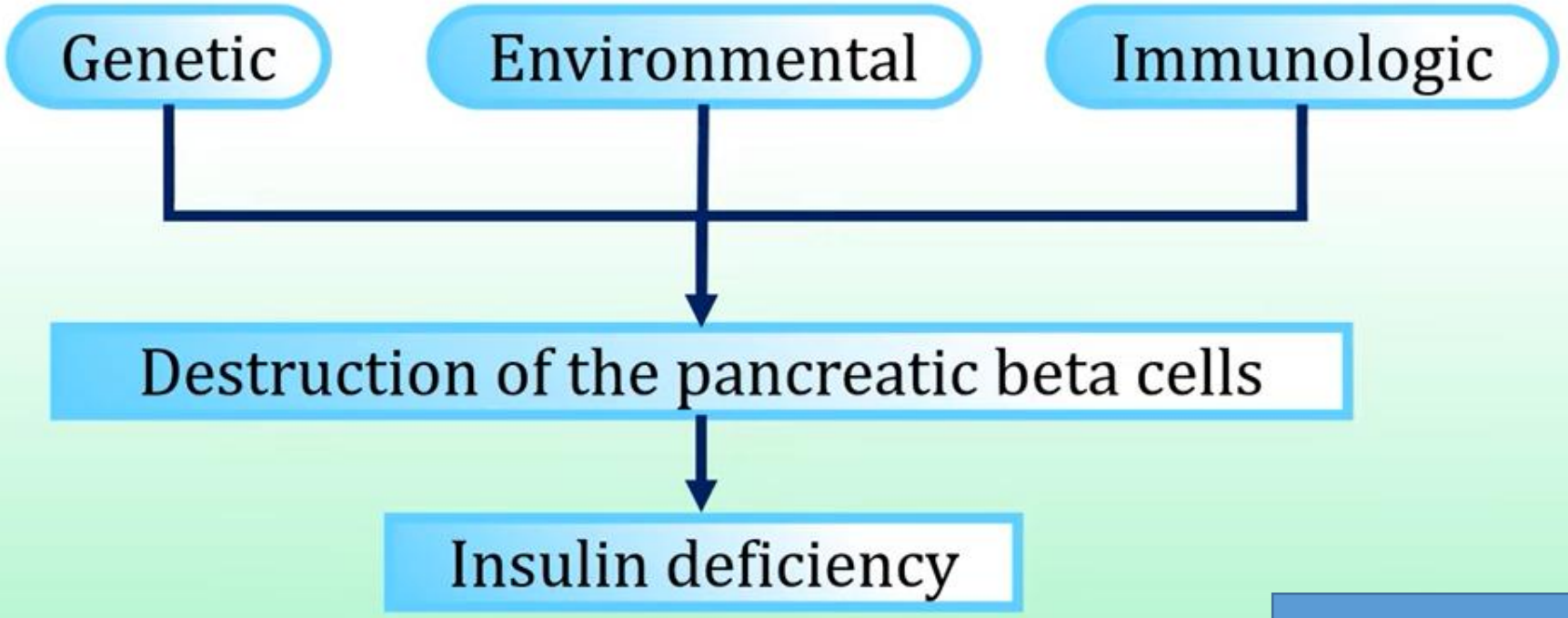
- Most common form of diabetes in childhood, but it can present at any age
- 10% of diagnosed diabetics
- Progressive decrease in insulin levels
- Most patients depend on exogenous insulin for survival
- Develop ketoacidosis in absence of insulin therapy
- Clinical manifestations occur late

Type 1 Diabetes: Pathogenesis

- Genetic susceptibility
- Class II MHC (HLA-DR) genes
- HLA-DR3, or DR4 or both

- Viral Infections
- (mumps, rubella,
- coxsackie B viruses)

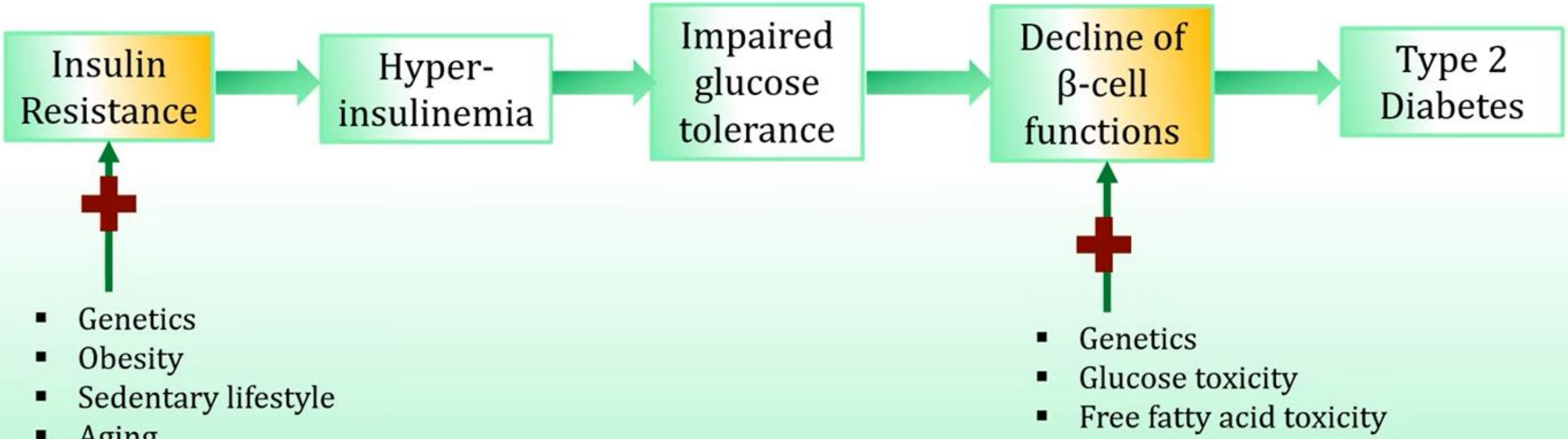
- Failure of self-tolerance in T cells specific for beta cell antigens
- Autoantibodies against a beta cell antigens (insulin, glutamic acid decarboxylase-GAD)



Type 2 Diabetes : Pathogenesis

- ✓ Insulin resistance alone will not lead to diabetes.
- ✓ It develops in insulin resistant individuals who also show impaired β-cell functions

IR: failure of target tissues to respond normally to insulin(Liver, Muscle, Adipose Tissues)



Reference: Lippincott's illustrated Reviews: Biochemistry(5th ed.pg 344)

Type 2 Diabetes : Obesity & Insulin Resistance

Adipocytes

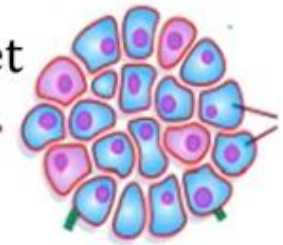
Adipokines

FFAs

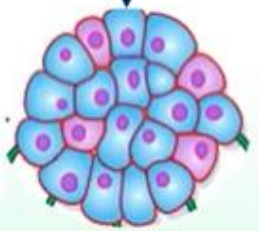
Inflammation

Insulin Resistance

Pancreatic islet Beta cells



Beta cells compensation



Beta cells failure

Insulin secretion by Beta cells	Blood glucose
Normal	Normal
Increased	Impaired Glucose tolerance
Decreased	Diabetes Mellitus

Pro-hyperglycemic

- ✓Resistin
- ✓TNF-alpha
- ✓IL-6
- ✓Retinol binding protein(RBP)

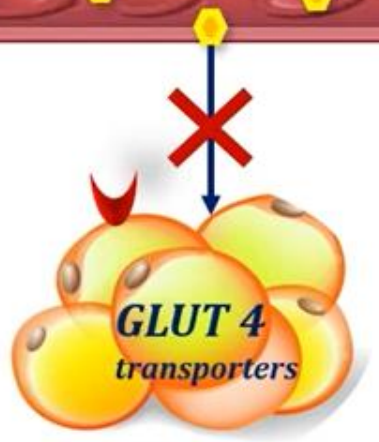
Anti-hyperglycemic

- ✓ Leptin
- ✓ Adiponectin
- ✓ Omentin
- ✓ Visfatin

Type 1 Diabetes: Metabolic Changes

Insulin

Insulin deficiency



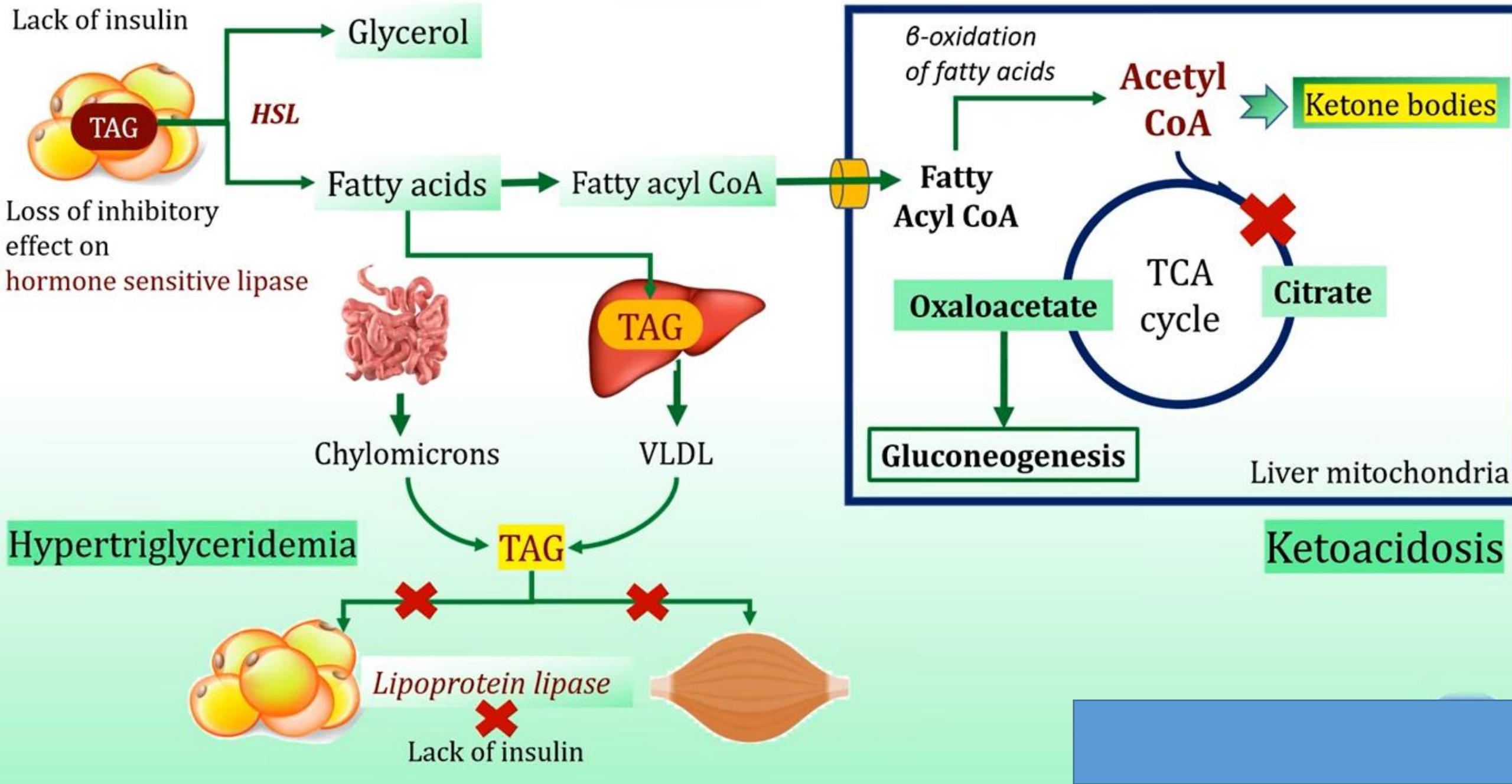
- ↓ Peripheral uptake of glucose
- ↓ Lipogenesis
- ↑ Lipolysis

- ↓ Peripheral uptake of glucose
- ↓ Glycogenesis
- ↑ Glycogenolysis
- ↑ Protein breakdown

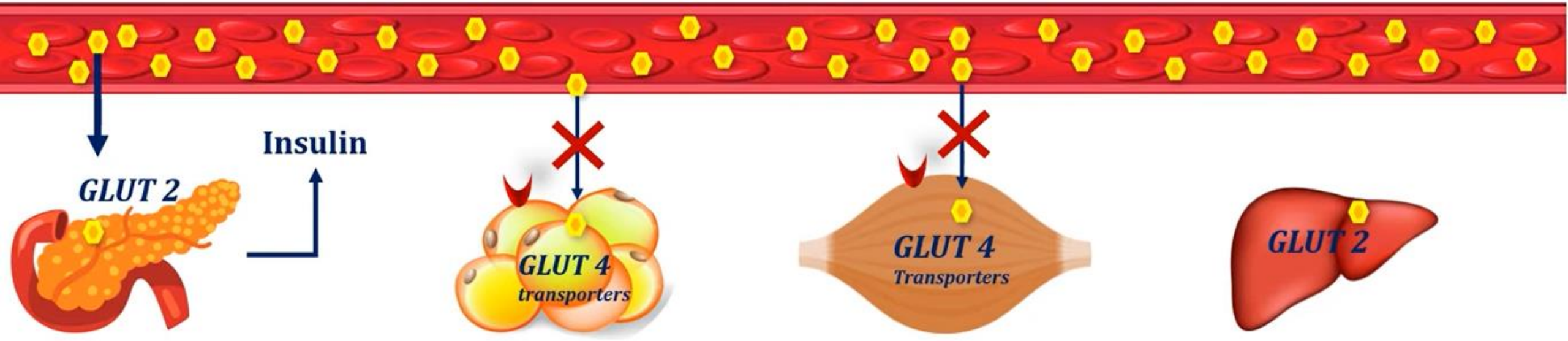
- ↓ Glycogenesis
- ↓ Lipogenesis
- ↑ Gluconeogenesis
- ↑ Glycogenolysis

↑ Glucose ↑ Free fatty acids → Acetyl CoA → Ketone Bodies ↑ TAG

Ketoacidosis & Hypertriglyceridemia in Type 1 Diabetes



Type 2 Diabetes: Metabolic Changes



↑ Glucose

↑ Free fatty acids

↑ TAG

Hyperglycemia

Dyslipidemia

Ketosis: Usually Minimal or absent

Diabetes: Clinical Features: Biochemical Basis

Hyperglycemia

Ketoacidosis

Glycosuria

Ketonuria

Polyuria

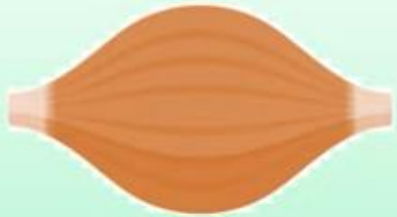
Volume depletion

Polydipsia

Polyphagia

Lipolysis

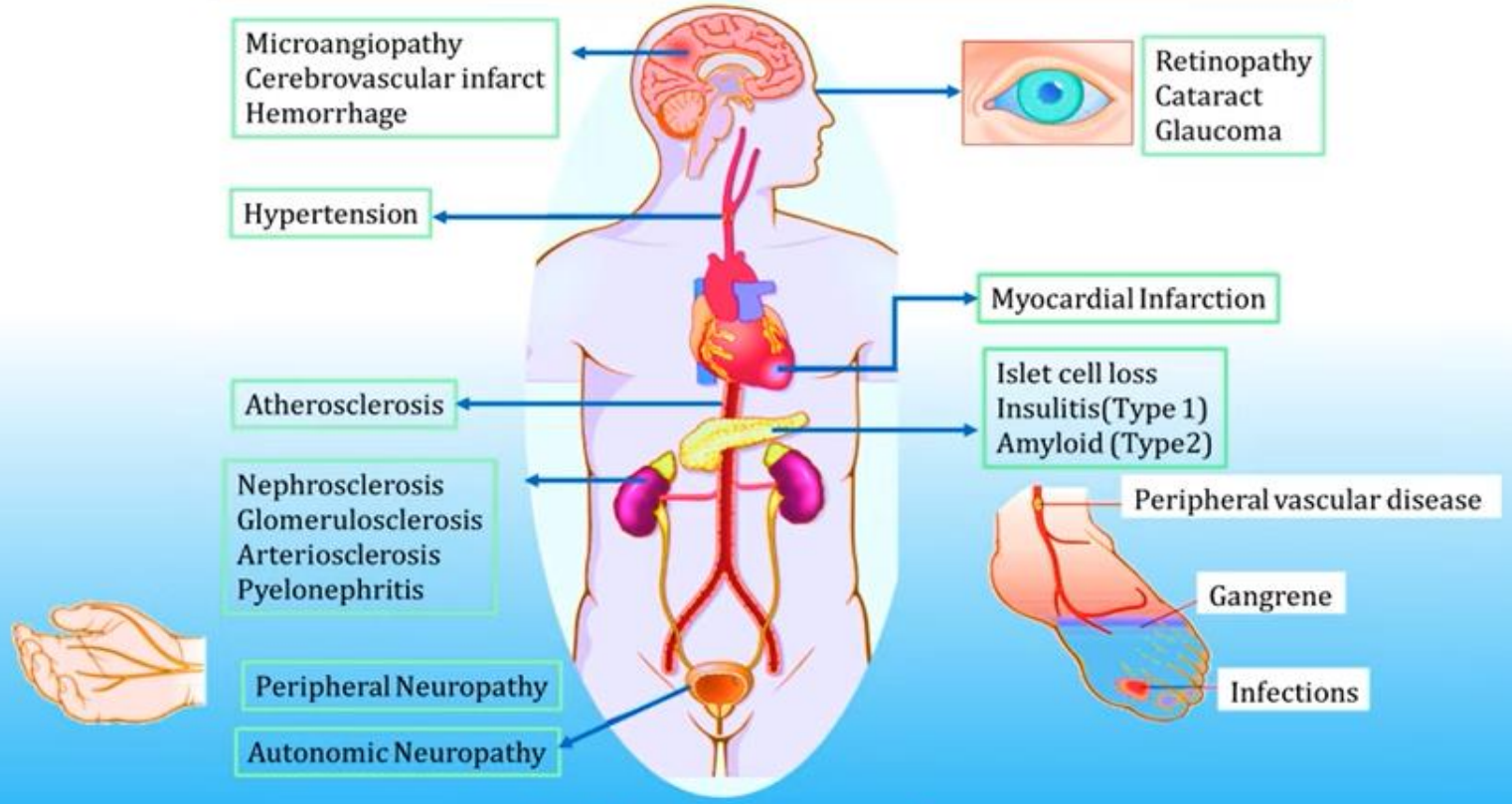
Protein catabolism



Type 1 Vs Type 2 Diabetes Mellitus

	Type 1 DM	Type 2 DM
Age of onset	Childhood & adolescence	Usually adulthood
Nutritional status	Normal weight or weight loss	Obesity (80%)
Prevalence	10% of diagnosed diabetics	90% of diagnosed diabetics
Genetic	Linkage to MHC class II genes, HLA DR3 and/or DR4	No HLA Linkage
Acute complications	DKA in absence of insulin therapy	Hyperglycemic hyperosmolar coma
Plasma Insulin	Progressive decrease in insulin levels	Increased blood insulin (early), normal or moderate decrease in insulin (late)
Pathogenesis	Genetic, Environmental, immunologic factors	Insulin resistance in peripheral tissues, Multiple obesity associated factors linked to IR
Pathology	Autoimmune insulitis	Amyloid deposition in islets(late)
	Beta cell depletion, islet atrophy	Mild beta cell deletion

Diabetes-Complications Mechanisms



Diabetes: Complications

Acute

- Diabetic Ketoacidosis(DKA)
- Hyperosmolar hyperglycemic state (HHS)

Chronic

Microvascular

- Diabetic Retinopathy
- Diabetic Nephropathy (Diabetic Kidney disease)
- Diabetic Neuropathy

Macrovascular

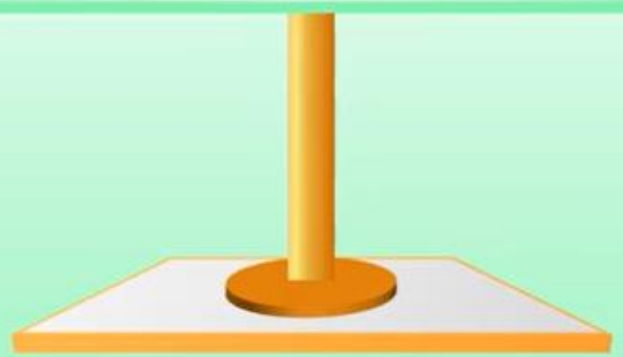
- Coronary heart disease,
- Cerebrovascular disease
- Peripheral vascular disease

Others

- Cataract, Glaucoma
- Infections, Foot ulcers
- Skin infections
- fungal infections

Diabetes ketoacidosis Vs Hyperglycemic Hyperosmolar state

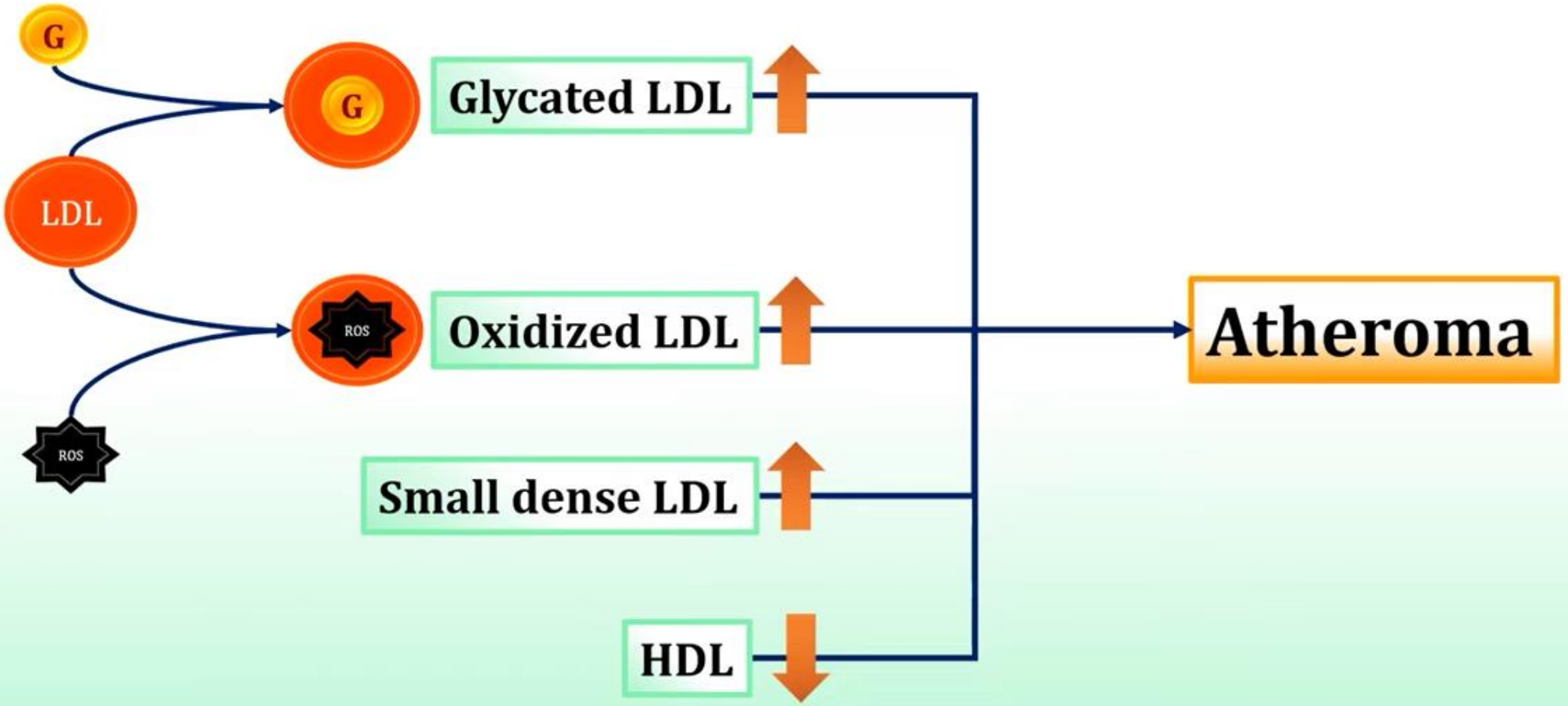
- More common in Type 1 DM
- Hyperglycemia
- Dehydration
- Ketosis, Ketonuria
- Increased anion gap acidosis
- Hyperventilation, fruity odor
- Hyponatremia
- Hyperkalemia



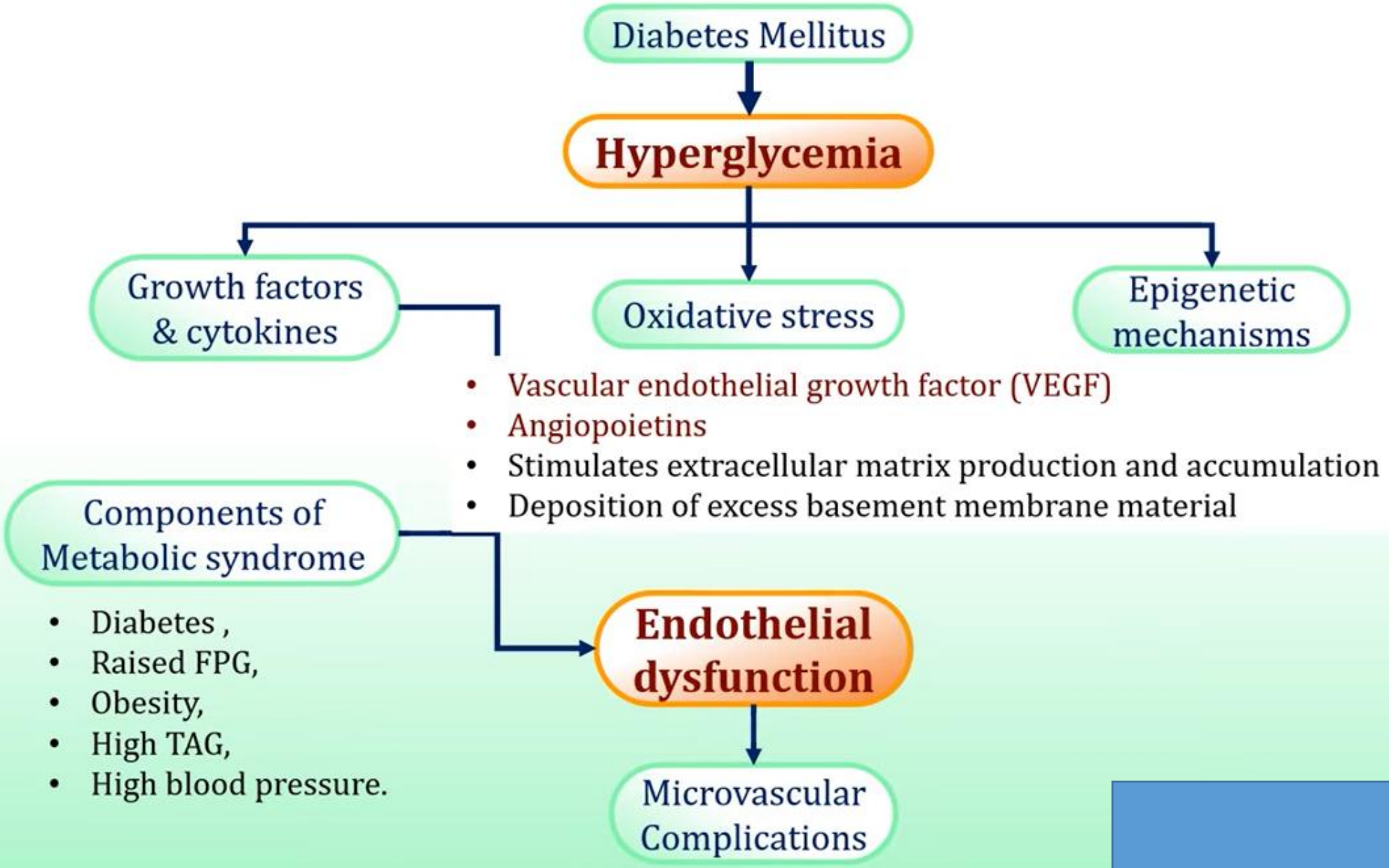
- More common in Type 2 DM
- Hyperglycemia
- Dehydration
- Increased plasma osmolarity
- No ketosis
- No ketonuria
- Normal anion gap
- No significant acidosis

Diabetes and Atherosclerosis: Macrovascular Complications

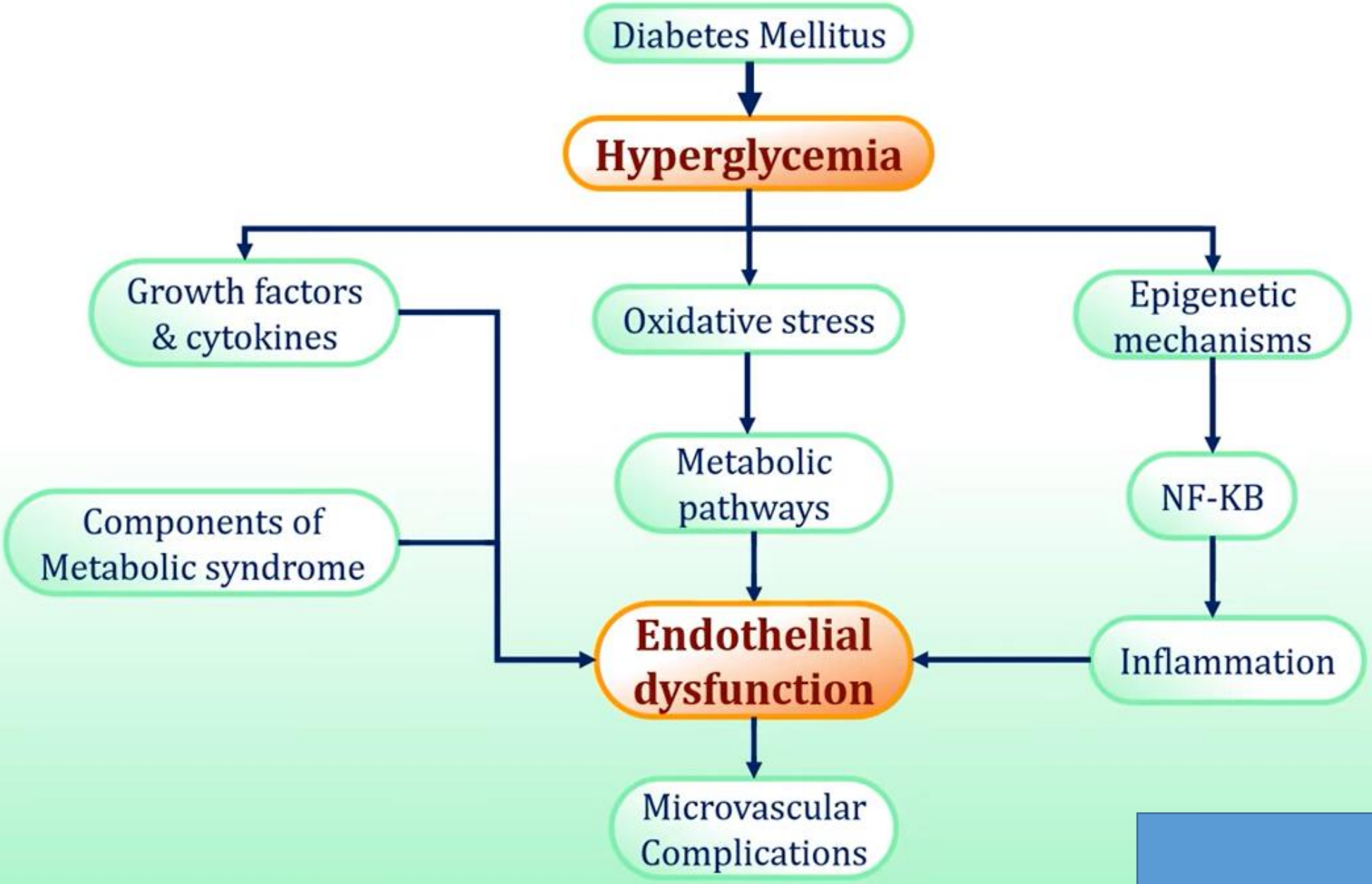
HYPERGLYCEMIA



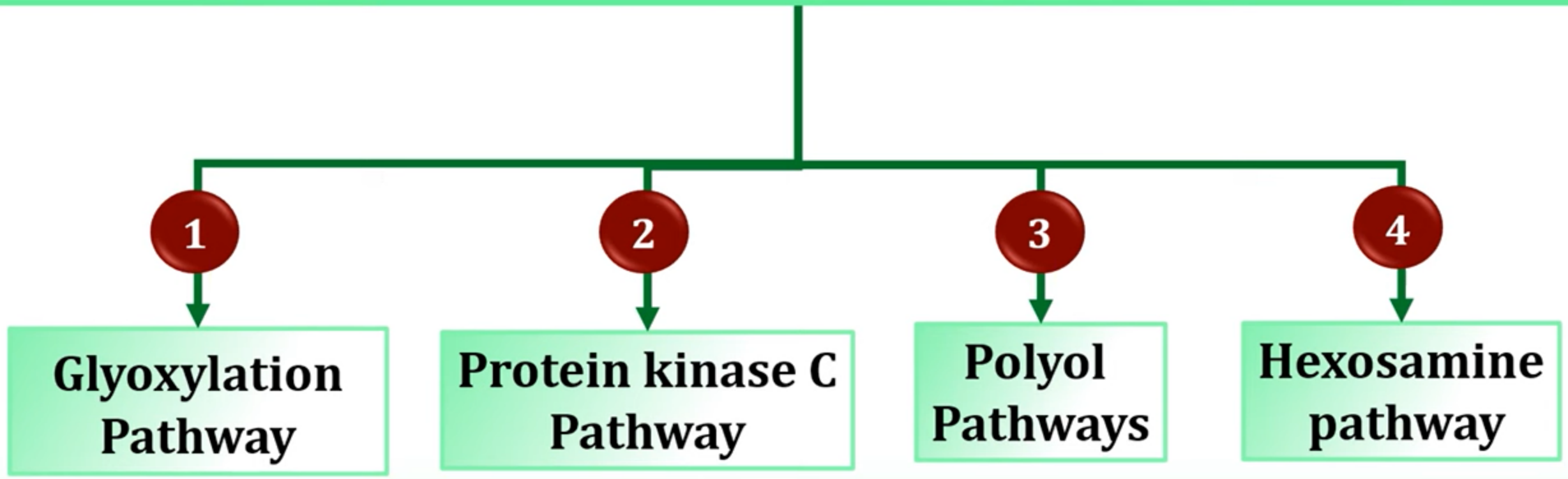
Molecular Mechanisms of Macro/Microvascular complications



Molecular Mechanisms of Macro/Microvascular complications



Hyperglycemia: Activation of Metabolic Pathways

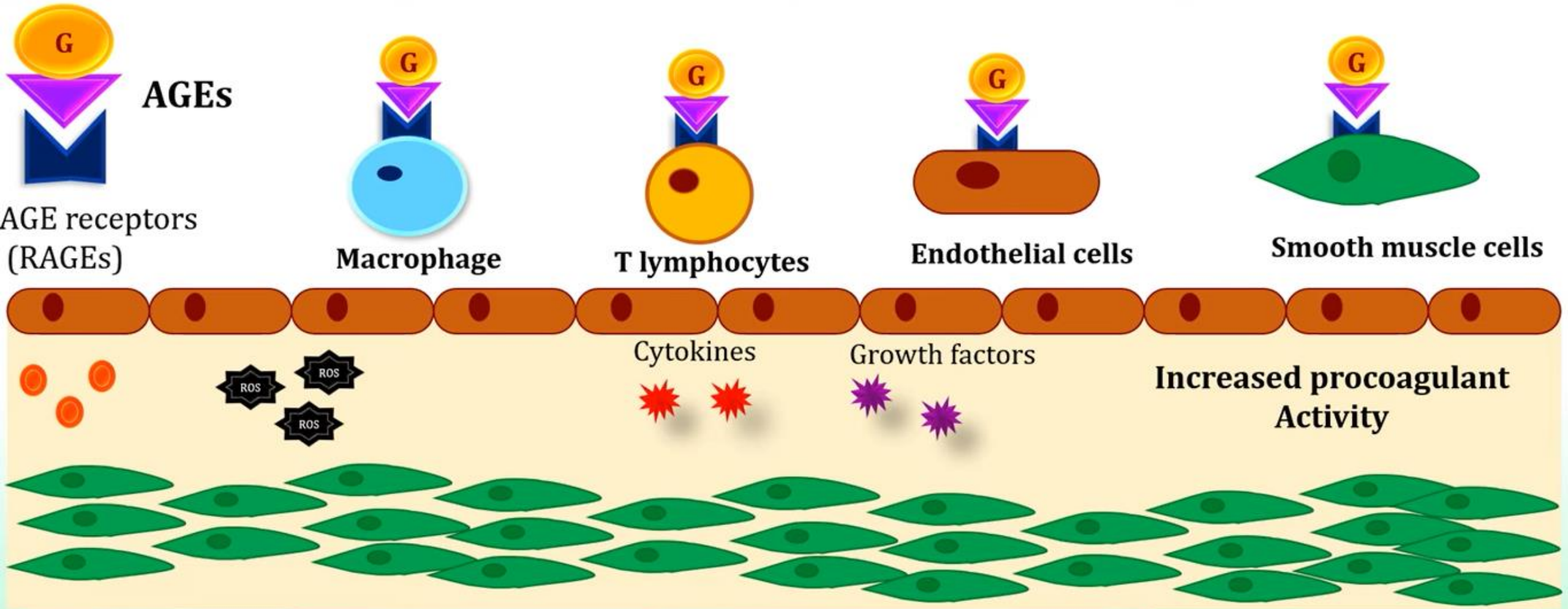


Advanced Glycation end products (AGEs)

Modification of

- Intracellular protein
- Extracellular matrix protein and components
- Plasma protein

1: Glyoxylation pathway: Advanced Glycation End Products (AGEs)



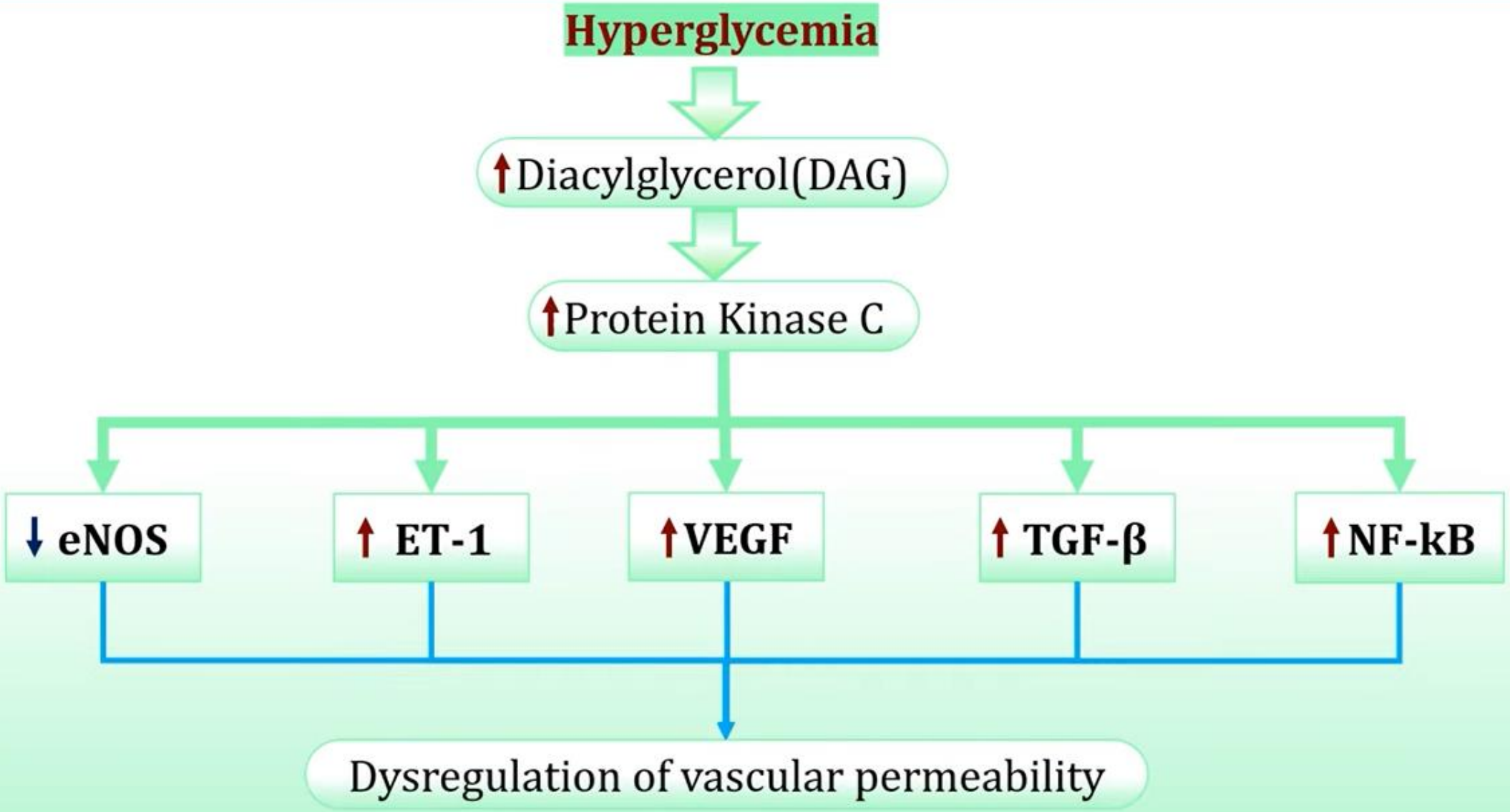
Trapping of LDL

Generation of reactive Oxygen species (ROS)

Release of cytokines, growth factors (TGFβ),VEGF

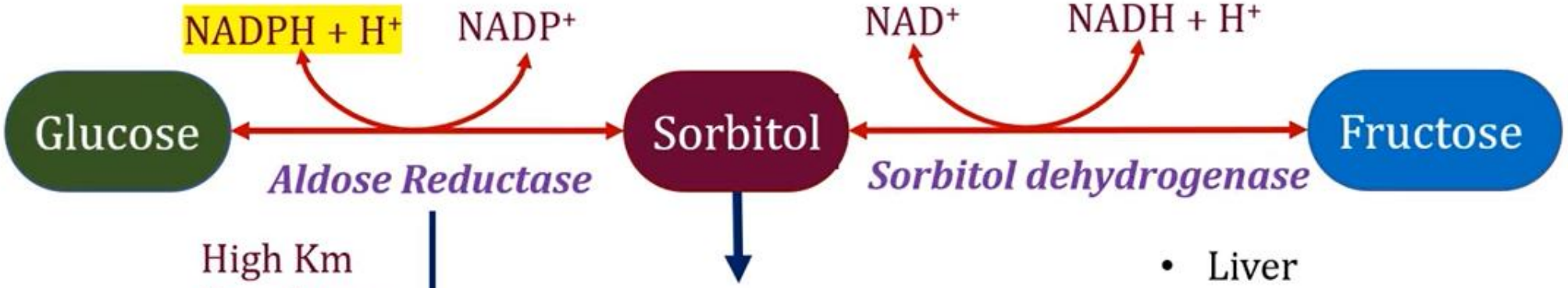
Proliferation of Vascular smooth muscle cells & Synthesis of ECM

2: Activation of Protein kinase C



3: Polyol pathways

- Polyol pathway: Sorbitol is a polyhydric sugar alcohol



High Km
For glucose

Activation of pathway in DM

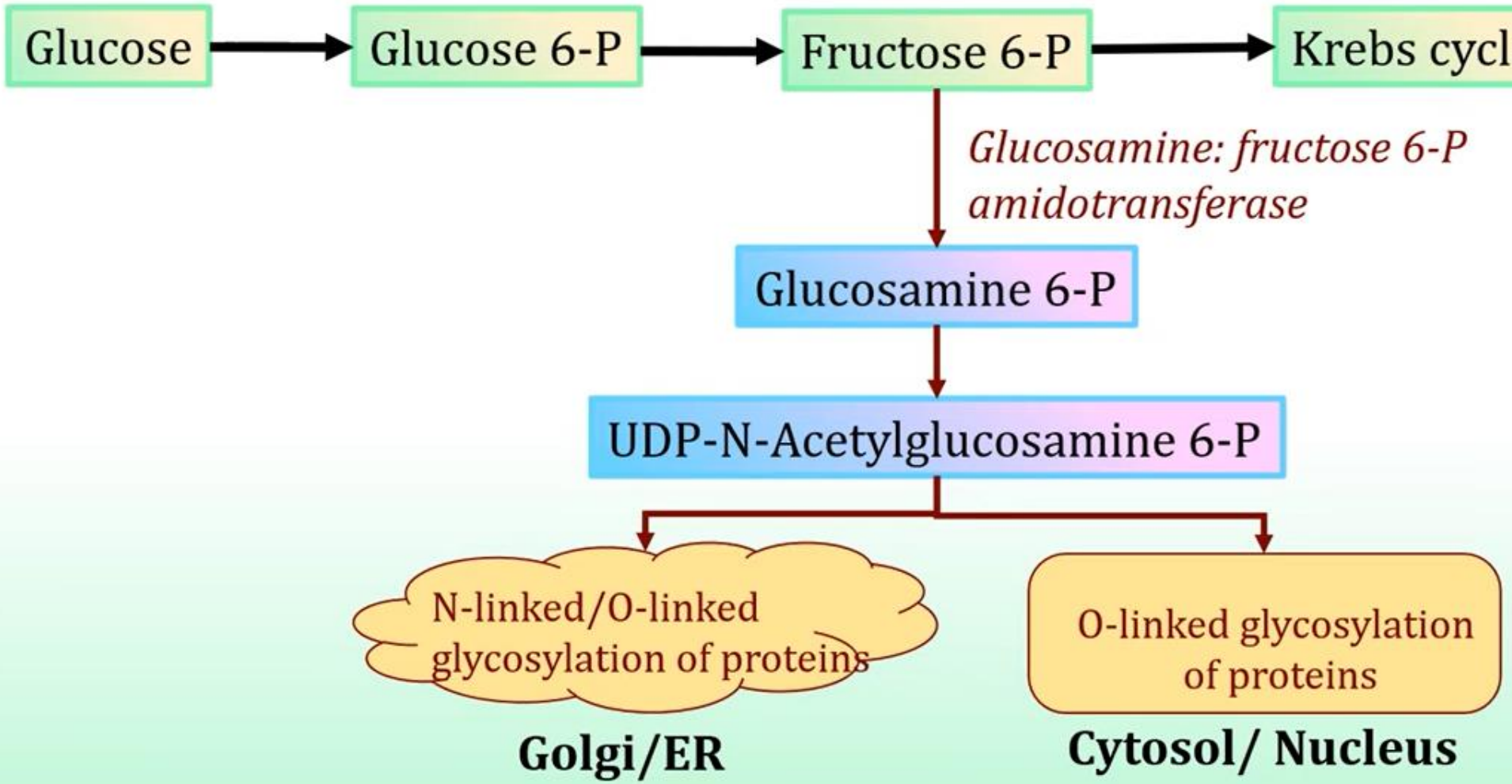
- Eye lens,
- Kidneys,
- Nervous tissues

(Schwann cells of the nerve sheath)

- Liver
- Seminal vesicles
- (Fructose-Fuel of spermatozoa)

- Cataract
- Nephropathy
- Neuropathy

4: Hexosamine Pathway



- Increased expression of TGF-β
- Modification of eNOS(Nitric oxide synthase)

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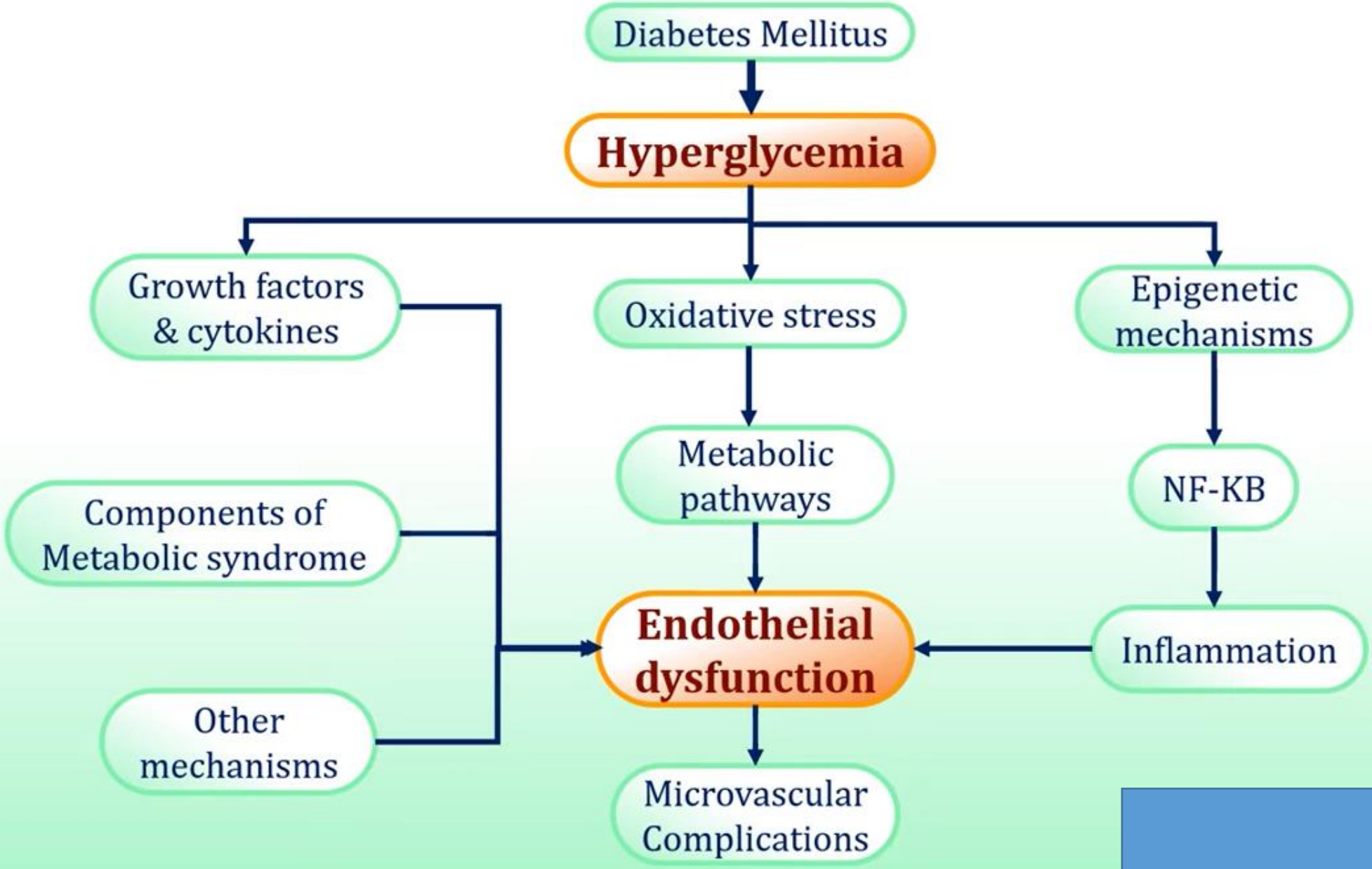


Diabetes: Diagnosis

	Normal	Prediabetes	Diabetes
HBA_1C	< 5.7 %	≥ 5.7 % - 6.4%	≥ 6.5 %
FPG	< 100mg/dl	≥ 100 mg/dl -125 mg/dl	≥ 126 mg/dl
		Impaired Fasting Glucose(IFG)	
OGTT 2 h PG	< 140 mg/dl	≥ 140 mg/dl - 199 mg/dl	≥ 200 mg/dl
		Impaired Glucose Tolerance(IGT)	

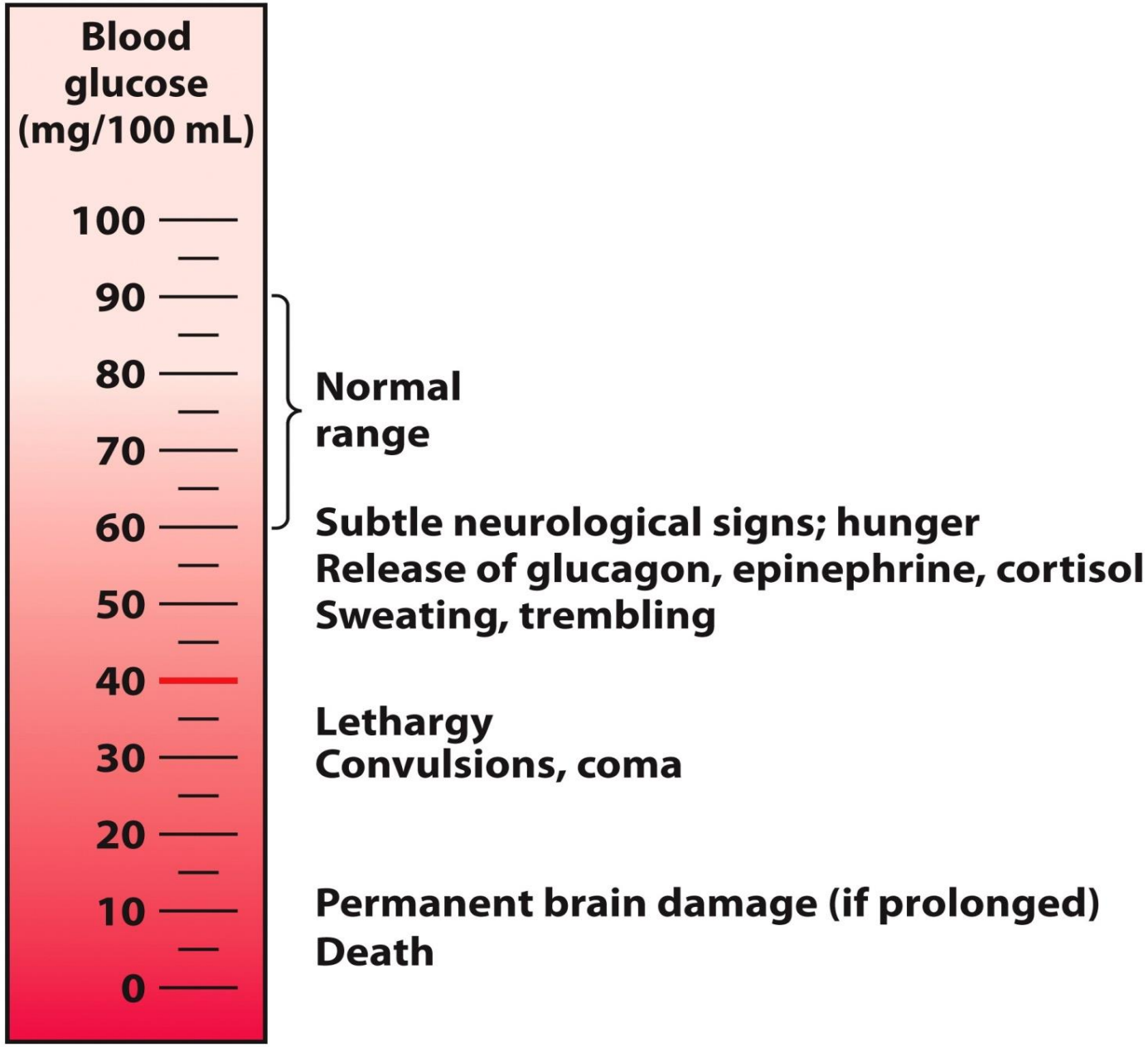
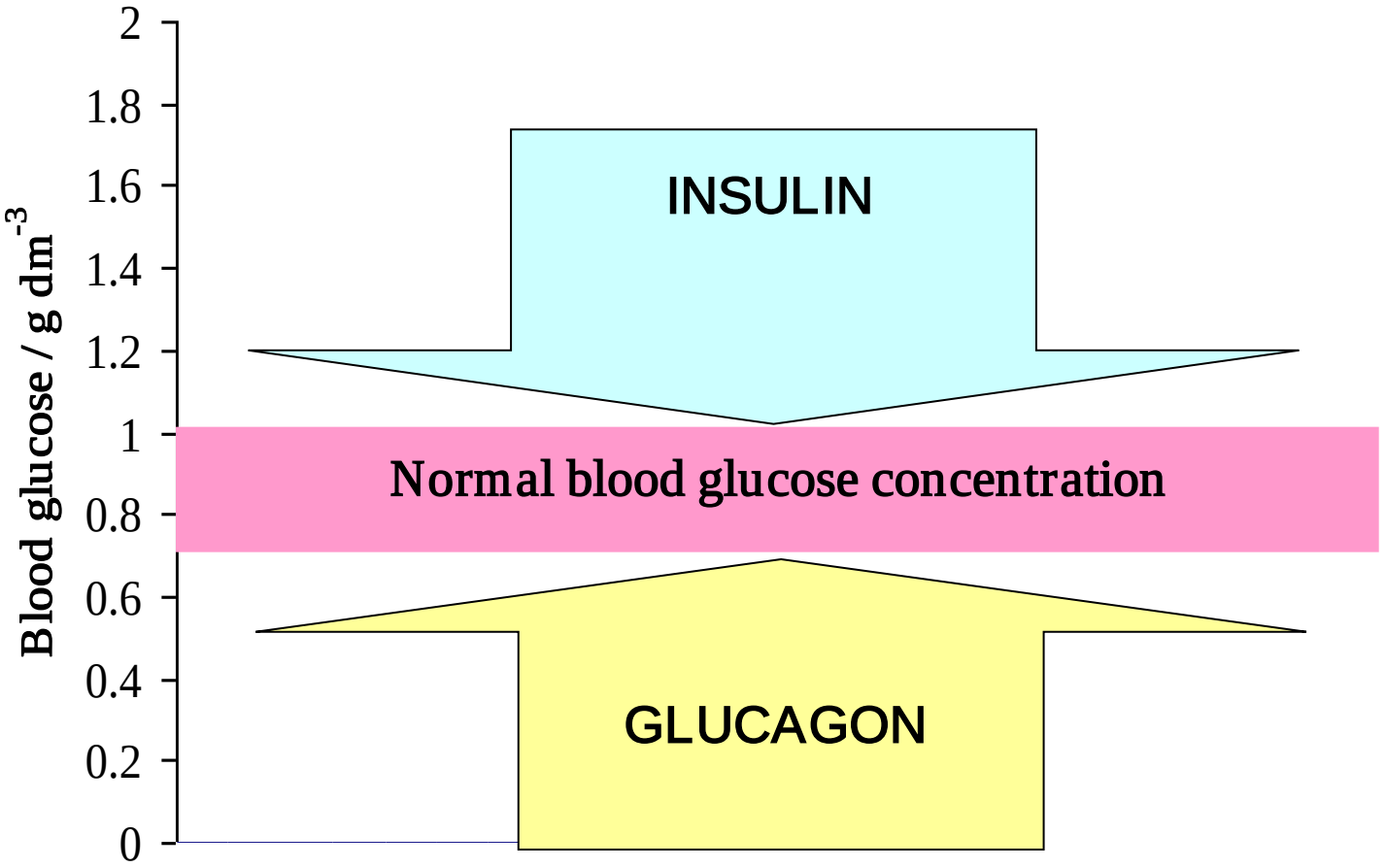
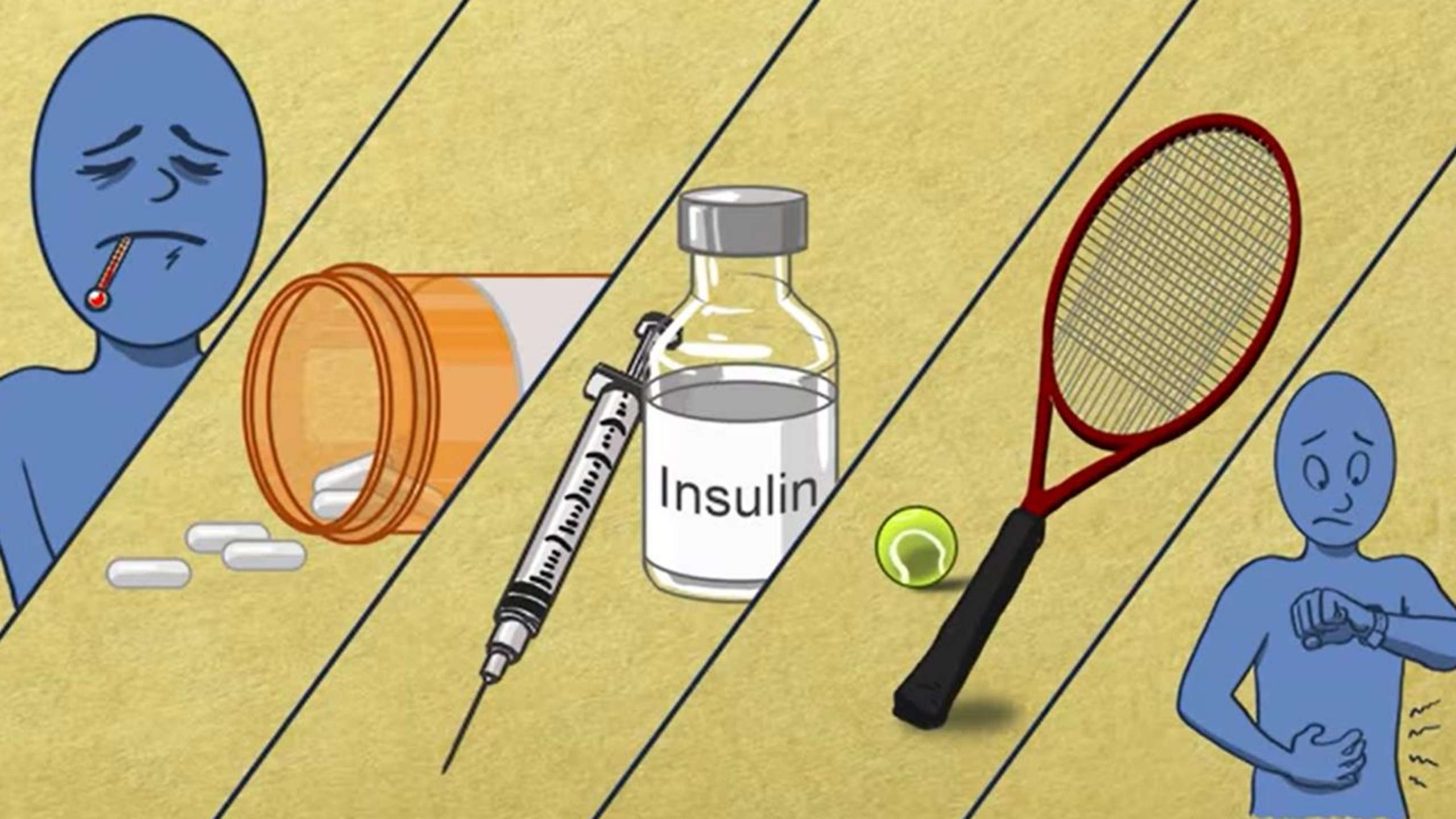


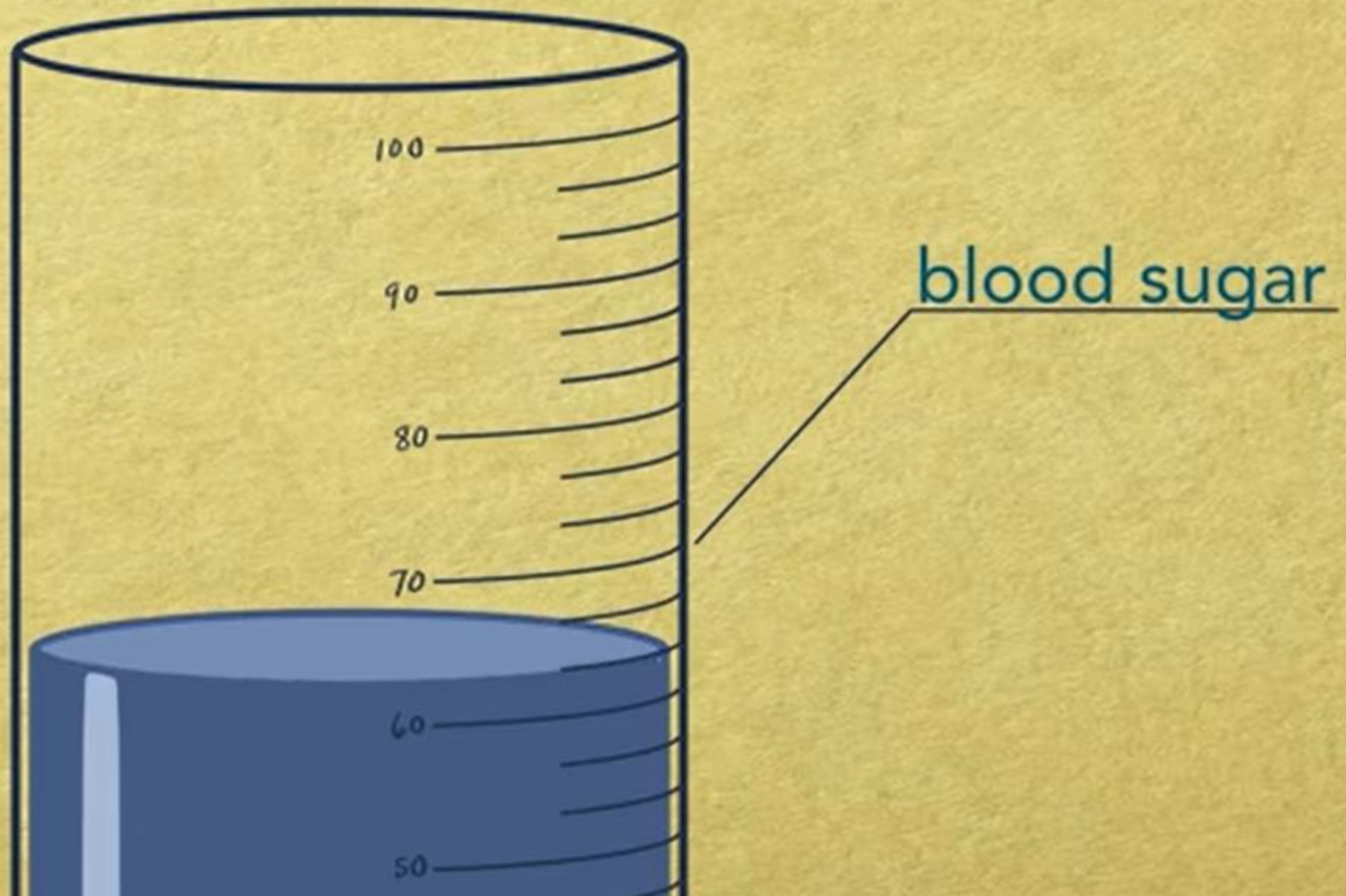
Figure 23-24
Lehninger Principles of Biochemistry, Sixth Edition

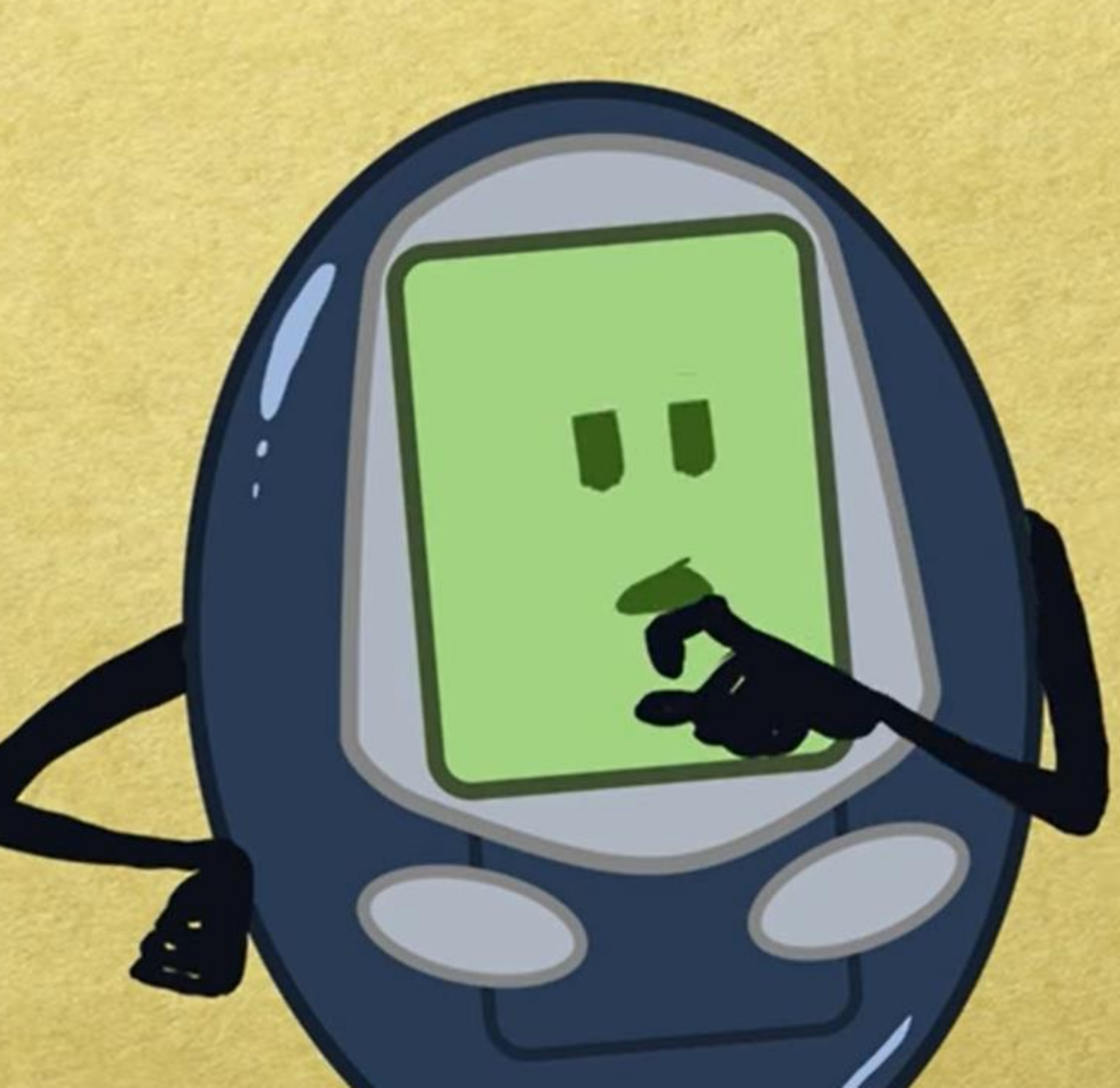
Summary



Hypoglycemia: Its Definition, Identification, Prevention, and Treatment

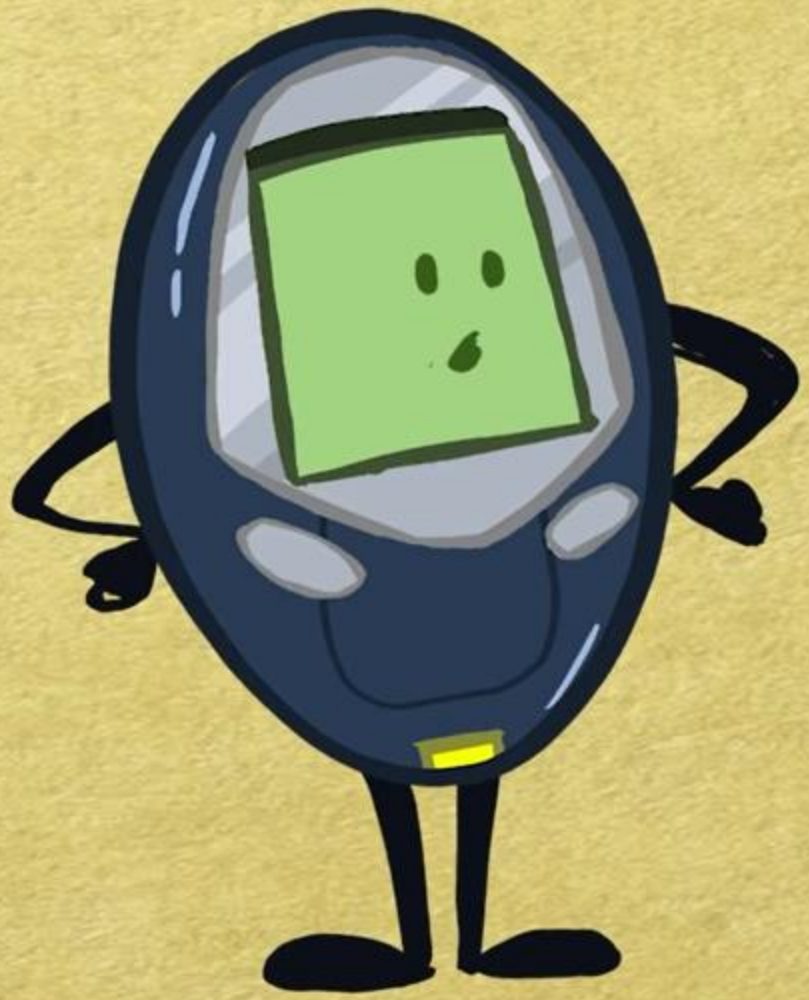

Insulin


100
90
80
70
60
50
blood sugar

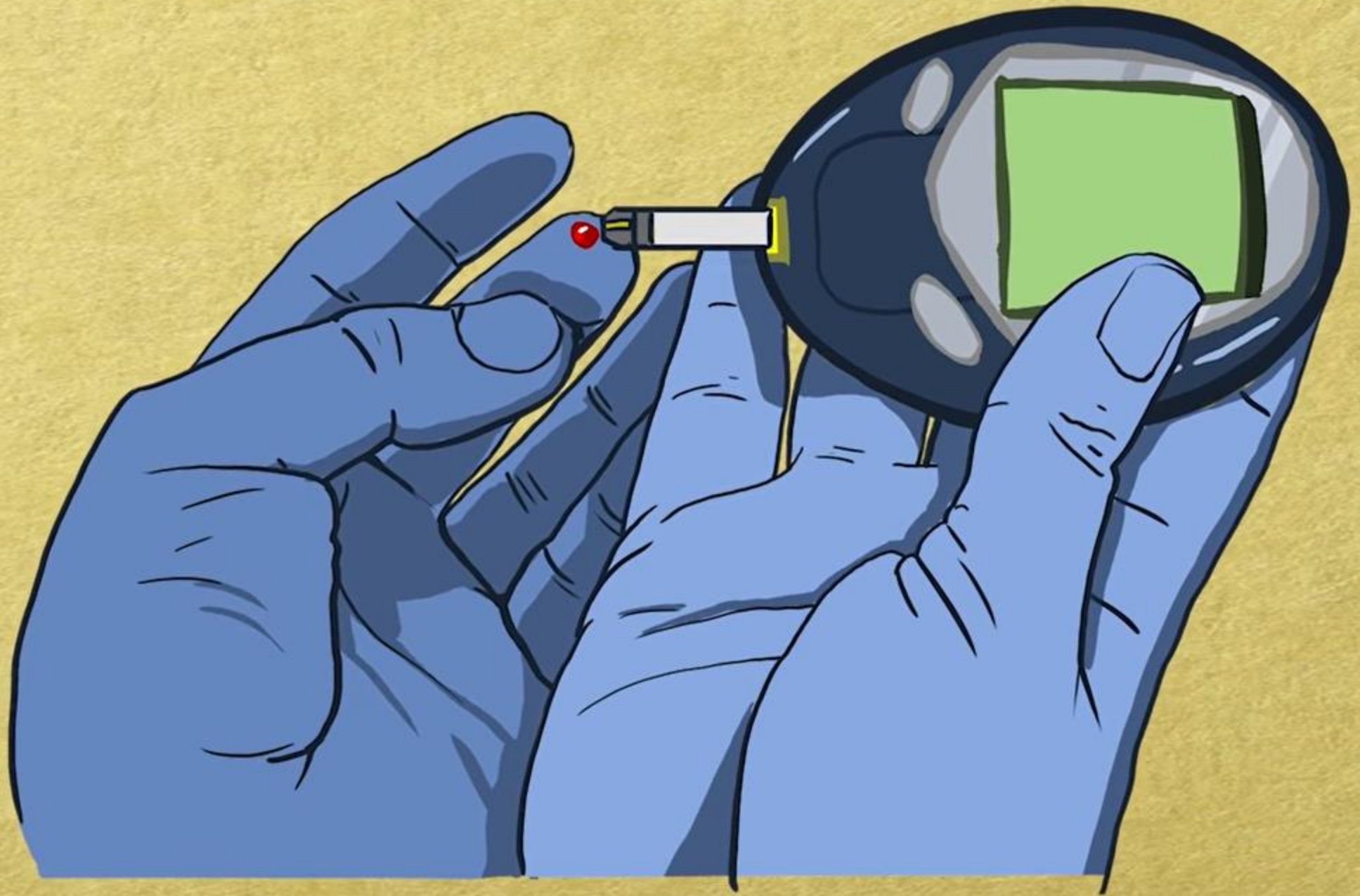


What is the typical range for people with diabetes?

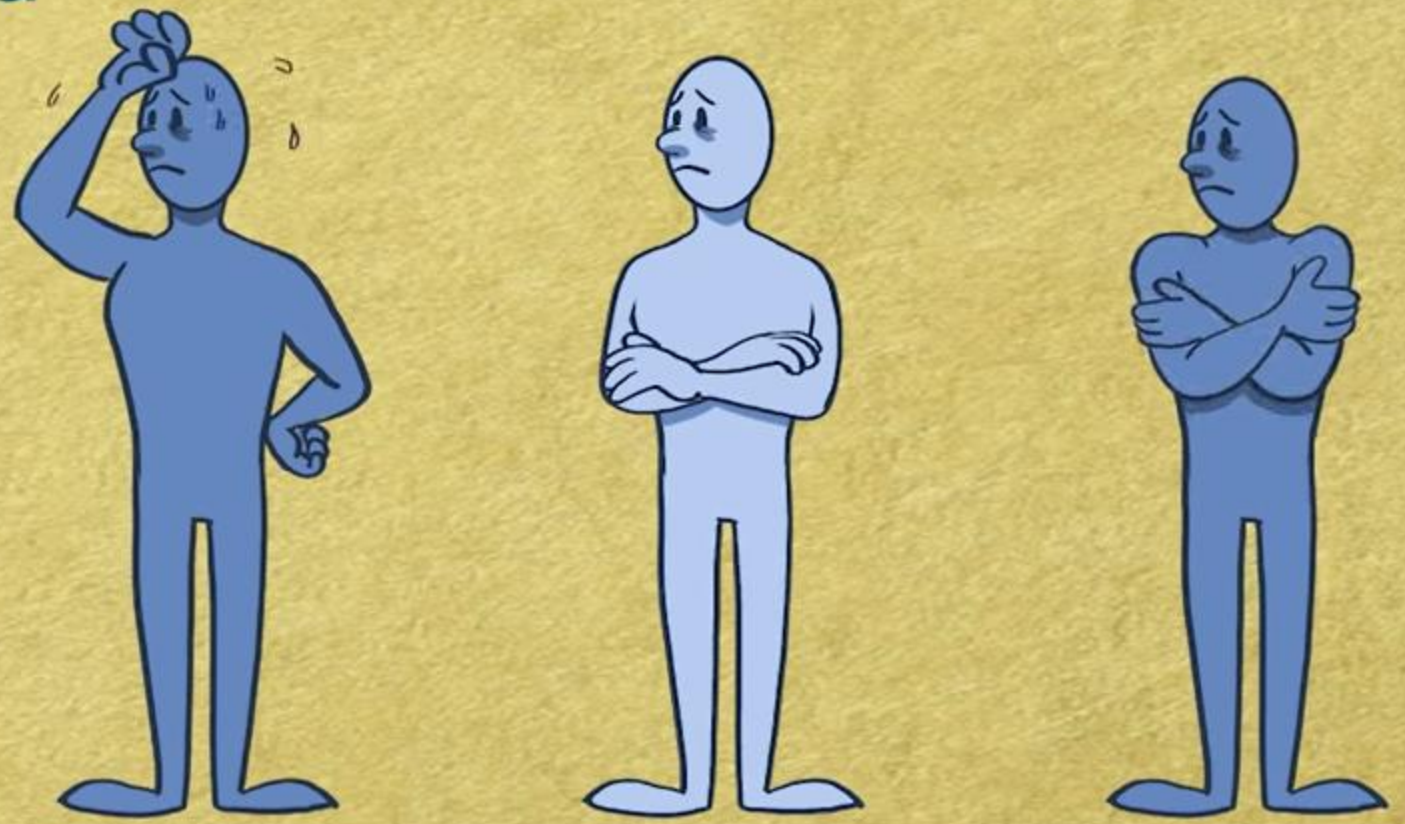
Goals for a Person with Diabetes	*Blood Sugar Levels in mg/dL*
Fasting (8 hours with no food or drink except water)	*80 - 130*
2 hours after eating	*< 180*



How do you know
if you have
low blood sugar?



- dizzy or light-headed
- confused or disoriented
- blurred vision
- fast or irregular heartbeat
- vivid dreams, nightmares, or sudden awakenings



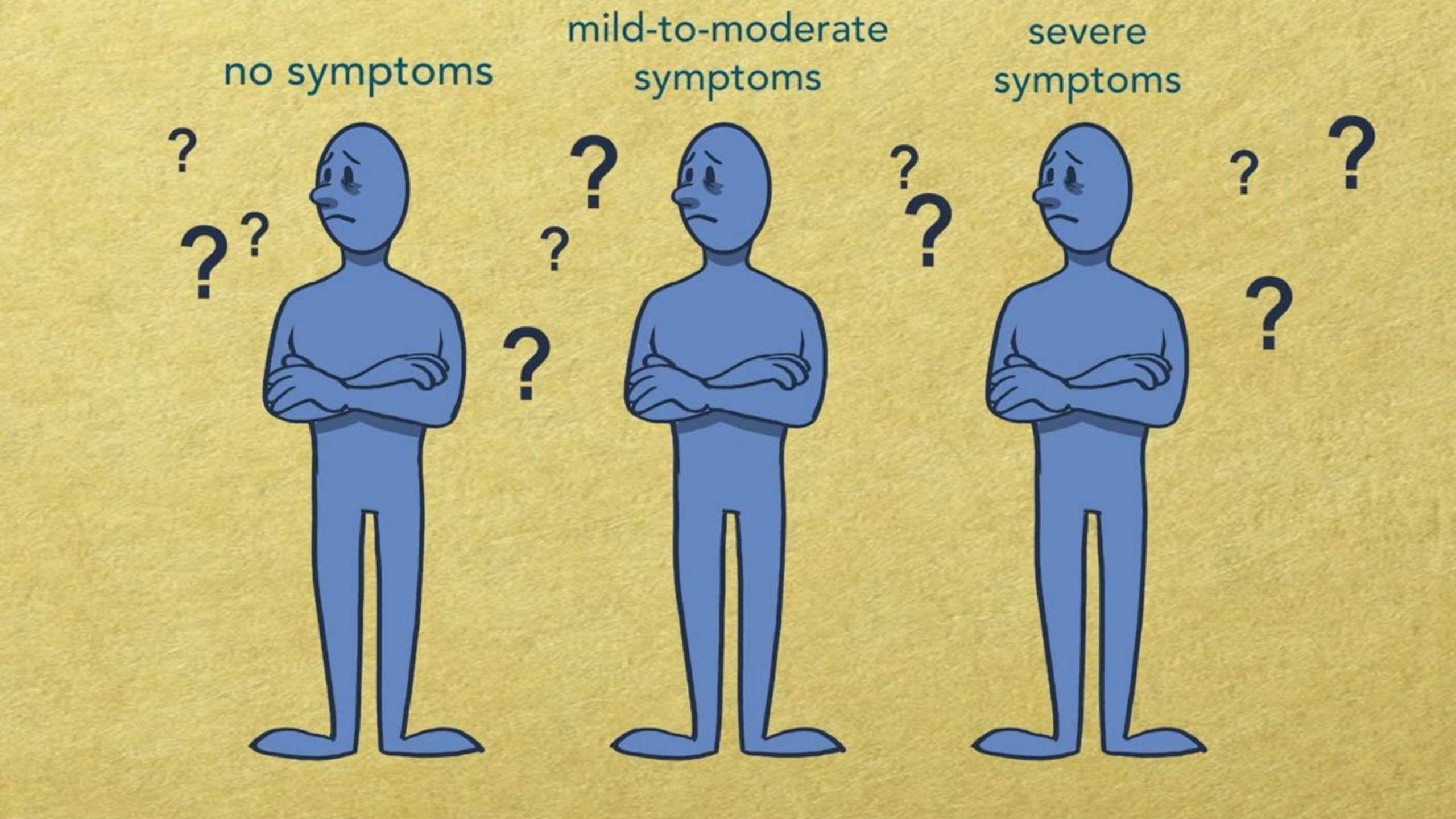
Severe Symptoms

- unable to eat or drink
- having seizures, convulsions, or jerky movements
- falling unconscious

Hypoglycemic Unawareness

Hypoglycemic Unawareness

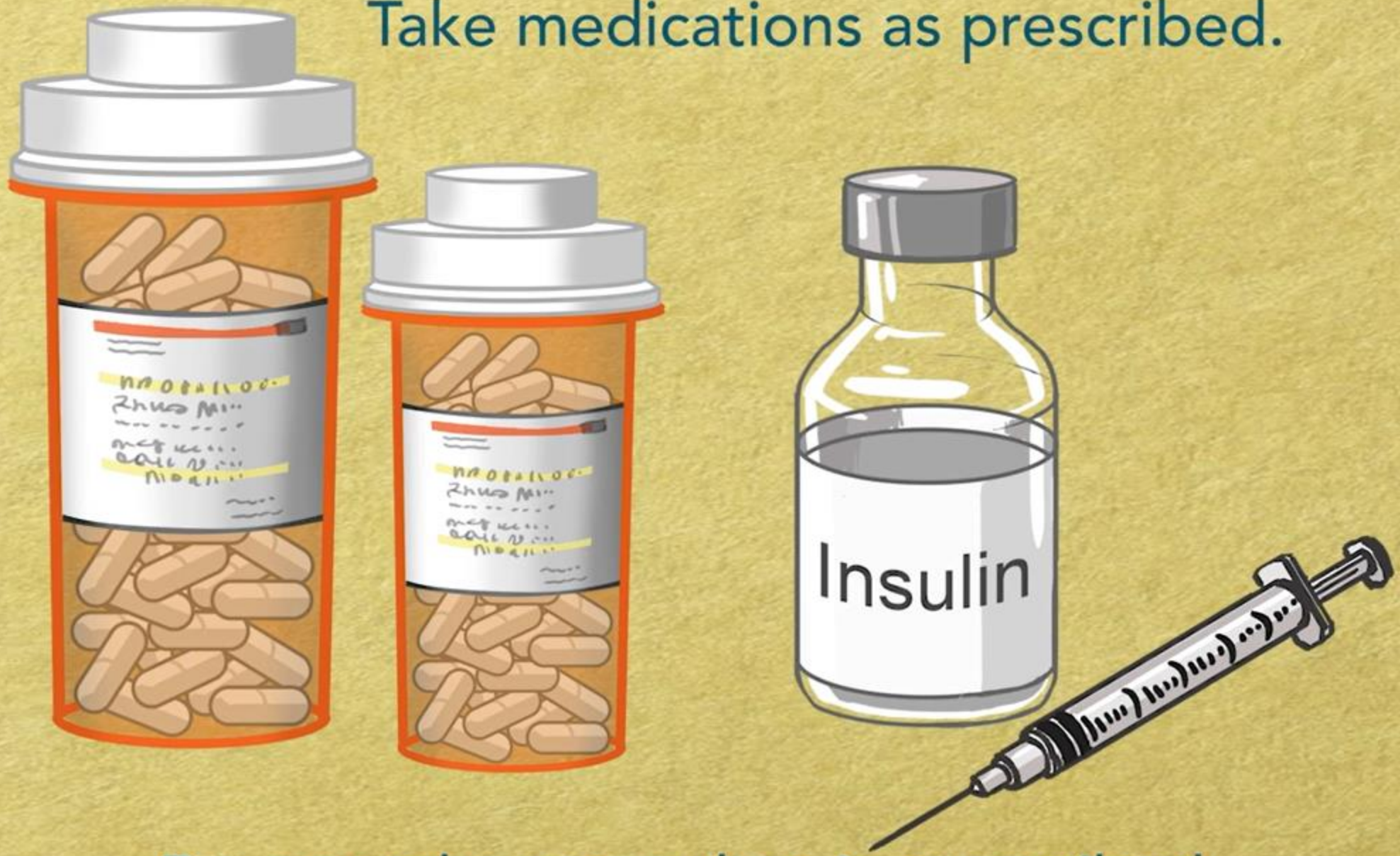
DANGER!
DANGER!


no symptoms
mild-to-moderate symptoms
severe symptoms

The ONLY way to TRULY know if you have low blood sugar is to check with an FDA-approved ✓ glucose meter.

How do you prevent hypoglycemia?

Take medications as prescribed.



Do ***not*** take more than is prescribed.

Taking ***more*** than is prescribed is ***dangerous*** and could lead to hypoglycemia.

Check your blood sugar regularly

How do you treat hypoglycemia?

Glucometer Reading of Blood Sugar Levels in mg/dL	Recommended Actions
59 - 70	Eat 15 grams of fast-acting carbohydrates and re-check blood sugar in 15 minutes
If after 15 minutes, your blood sugar is still below 70	Eat another 15 grams of fast-acting carbohydrates
80 - 130	Once your blood sugar is in the normal range, eat a small snack to prevent another low.


diet
SODA
8 oz.
skim
milk
glucose
tablets
4 oz.
fruit
juice
glucose
gel
4 oz. regular
soda
15 grams


chocolate
bar
whole
or
2%
milk

What happens if your blood sugar goes too low and you are asleep or unresponsive?

Somebody who finds
you unresponsive
can easily inject
GLUCAGON
into your body.

GLUCAGON

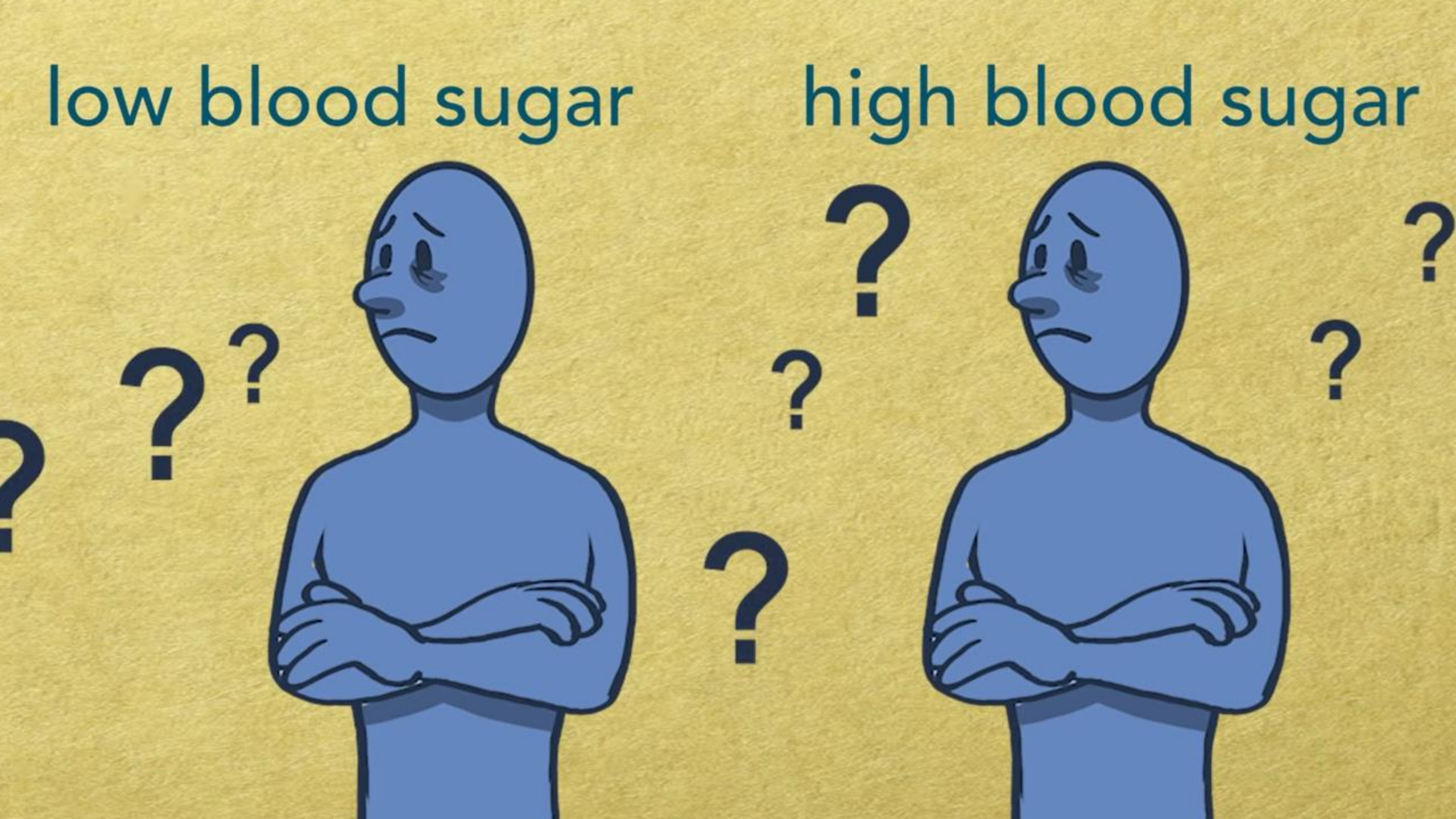
hypoglycemia
preventable

What is hypoglycemia?

Hypoglycemia means low blood sugar.

low blood sugar

high blood sugar



INVESTIGATIONS IN CASE OF DIABETES MELLITUS

- **Diabetes mellitus (DM):**

  Group of metabolic disorders sharing common feature of hyperglycemia.

- One of the top 10 "killers" in the US.

- Associated increased morbidity and mortality

Role of Lab Tests

- Diagnosis of DM
- Screening of DM
- Assessment of glycemic control
- Assessment of associated long-term risks
- Management of acute metabolic complications.

Diagnostic criteria for diabetes (ADA & WHO)

- A **fasting** plasma glucose ≥ **126 mg/dL**,
- A **random** plasma glucose ≥ **200 mg/dL** (in a patient with classic hyperglycemic signs),
- 2-hour plasma glucose ≥ **200 mg/dL** during oral glucose tolerance test (OGTT) with loading dose of 75 gm
- A glycated hemoglobin (**HbA1C)** level ≥ **6.5%**

IMPAIRED GLUCOSE TOLERANCE (PREDIABETES)

- A fasting plasma glucose between 100 and 125 mg/dL ("impaired fasting glucose"),
- 2-hour plasma glucose between 140 and 199 mg/dL following a 75-gm glucose OGTT, and/or
- A glycated hemoglobin (HbA1C) level between 5.7% and 6.4%

Diagnostic criteria for diabetes (ADA & WHO)

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- A **random** plasma glucose ≥ **200 mg/dL** (in a patient with classic hyperglycemic signs),
- 2-hour plasma glucose ≥ **200 mg/dL** during oral glucose tolerance test (OGTT) with loading dose of 75 gm
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Tests

- Blood gluocse
  - Fasting/ PP
  - Random
  - GTT
- Urine for sugars & ketones
- Other tests
  - HbA1C
  - Insulin assay
  - Islet auto-antibodies
  - Lipid Profile
  - Micro-albumin

Blood glucose estimation

- can be estimated in whole blood (capillary or venous blood), plasma or serum.
- Sodium fluoride bulb used to prevent glycolysis
- Plasma commonly preferred as WB glucose affected by conc of proteins
- Plasma gluocose levels 15% higher than whole blood glucose

Types of specimens

- **Fasting blood glucose:**
  - Blood Sample withdrawn after an overnight fast (no caloric intake for at least 8 hours).
- **Post meal or postprandial blood glucose:**
  - Blood sample collected 2 hours after the subject has taken a normal meal.
- **Random blood glucose:**
  - Blood sample is collected at any time of the day, without attention to the time of last food intake.

Methods for estimation

- Enzymatic methods:
  - Glucose oxidase-peroxidase
  - Hexokinase
  - Glucose dehydrogenase
- Chemical methods:
  - Orthotoluidine method
  - Blood glucose reduction methods using neocuproine, ferricyanide, or copper.

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Oral Glucose tolerance test

- Used for diagnosis of DM & screening n diagnosis of gestation DM
- Screening recommended at 24-28 weeks of gestation in women who were not previously diagnosed with overt diabetes
- Patient preparation in OGTT (recommended by WHO)
  - Test in morning
  - Fasting (no caloric intake for ≥ 8 hours)
  - On day of test, discontinue medications which affect carb metabolism
  - 3 days of unrestricted diet (≥ 150 gms carb per day)
  - Normal physical activity
  - No smoking during the test

One-step diagnosis strategy	Two-step diagnosis strategy
• Perform 75-g OGTT with plasma glucose measurement • Test in the morning after the patient has fasted for ≥8 hours • Repeat test at 1 and 2 hours after initial measurement	Step 1: • Perform a 50-g nonfasting GLT with plasma measurement at 1 hour • If PG measured 1 hour after the load is ≥140 mg/dL (7.8 mmol/L), proceed to 100-g OGTT
Diagnosis is confirmed when PG levels meet or exceed: • Fasting 92 mg/dL (5.1 mmol/L) • 1 hr: 180 mg/dL (10.0 mmol/L) • 2 hr: 153 mg/dL (8.5 mmol/L)	Step 2: • Perform 100-g OGTT while patient is fasting Diagnosis is confirmed when two or more PG levels meet or exceed: • Fasting: 95 mg/dL or 105 mg/dL (5.3/5.8) • 1 hr: 180 mg/dL or 190 mg/dL (10.0/10.6) • 2 hr: 155 mg/dL or 165 mg/dL (8.6/9.2) • 3 hr: 140 mg/dL or 145 mg/dL (7.8/8.0)

Plasma glucose,mg/dL(mmol/L)

	Normal	Impaired	Diabetic
Fasting	<100	100-125	>/= 126
2-hr(OGTT)	<140	140-199	>/=200

Lab tests to Assess glycemic control

- HbA1c estimation
- Self monitoring of Blood Glucose
- Glycosuria (Urine examination)

HbA1c estimation

- To assess long term control (6-12 weeks)
- Glycated hemoglobin refers to hemoglobin to which glucose attached nonenzymatically & irreversibly;
- Its amount depends upon blood glucose level and lifespan of red cells.
- glycated hemoglobin level also correlates with the risk of the development of chronic complications
- Should be less than 7% in diabetics

SELF MONITORING OF BLOOD GLUCOSE (SMBG)

- For daily monitoring own blood glucose levels
- Insulin dosages can be adjusted
- Portable glucometers
- Devices measure capillary whole blood glucose obtained by fingerprick and use test strips that incorporate glucose oxidase or hexokinase
- May avoid major hypoglycemic attacks

URINE TEST

- Glucosuria
  - Benedict/ Strip

- Ketonuria (Rotheras test)

MICROALBUMINURIA

- Urinary excretion of 30-200mg/d of albumin
- Precedes other signs of ds. by 10-15 yrs
- Earliest indicator of diabetic nephropathy

Other tests

- Dyslipidemia in type II DM
  - Pt. with borderline high LDL cholesterol (130-160 mg/dL)- high risk of CHD
- Hormones
  - Insulin, C-peptide, glucagon
- Serological tests
  - Type I with islet cell autoAb, insulin autoAb

Diagnosis of Acute Complications

- Diabetic Ketoacidosis (DKA)
- Hyperglycemic hyperosmolar state (HHS)

- Hyperglycemia
- Ketosis
- Insulin deficiency
- Volume depletion
- Metabolic acidosis

Laboratory evaluation consists

- Blood and urine glucose
- Blood and urine ketone
- Arterial pH, Blood gases
- Serum electrolytes (sodium, potassium, chloride, bicarbonate)
- Blood osmolality
- Serum creatinine and blood urea.

Diagnosis of Acute Complications

	DKA	HHS
Glucose (gm/dL)	250-600	600-1200
Creatinine	Slightly ↑	Moderately
Osmolality (mOsm)	300-320	300-380 mOsm
Ketones	++++	+/-
pH	6.8-7.3	>7.3
Anion gap	>12	Variable
Sr Bicarbonates	<15 mEq/L	>15 mEq/L

Charts- Clinical Cases

Case 1

- A 65 yr old asymptomatic female came for routine investigations. On examination, patient is obese without any obvious abnormality. Routine CBC, Random blood sugar and urine examination done.

- Lab Investigations-
  - Hb- **10 gm%**
  - Random sugar- **295 mg/dl**
  - Urine examination-
    - **Benedicts test positive.**

Case 2

- A 25 yr old male presented with burning micturation, increased frequency of micturation, unexplained fatigue and slight dizziness since last 1 month. On examination, patient is moderately built without any obvious abnormality.

- Lab Investigations-
  - Hb-**12.5 gm%**
  - Random sugar- **255 mg/dl**
  - Urine examination-
    - Benedicts test positive.
    - Urine microscopy- 20 -25 pus cells/ hpf

Repeat investigations after one week

- Fasting plasma glucose- **170 mg/dl**
- Postprandial plamsa glucose- **240 mg/dl**

Case 3

- A 45 yr old male IT professional came for routine annual medical examination. On examination he was having central type of obesity and his blood pressure was 150/90 mm of Hg. Routine CBC, Random sugar and urine examination advised.

- Lab Investigations-
  - Hb-**13.5 gm%**
  - Random sugar- **165 mg/dl**
  - Urine examination- No abnormality detected.

Repeat investigations after one week

- Fasting plasma glucose- **115 mg/dl**
- Postprandial plamsa glucose- **170 mg/dl**

Case 4

- A 57 yr old female was brought to the emergency in semicomatose condition. She was immediately transferred to ICU and blood samples were taken for routine examination after primary management.

- Lab Investigations-
  - Hb- 8.5 gm%
- ABG showed
  - Blood pH- 7.1
  - Blood ketones- +++
  - Sr bicarbonates- 14 mEq/L

  - Random sugar- 375 mg/dl
  - Hb1Ac- 11%

- Urine examination-
  - Benedicts test- Positive.
  - Sulphosalycylic test- Positive.
  - Rotheras test- Positive

Case 5

- A 32 yr old pregnant female came for routine examination. She had a history of previous baby died at birth which was large for age weighing 4.5 kgs.

- Lab Investigations-
  - Hb- 8.5 gm%
  - PBS- Microcytic hypochromic
  - Urine examination- No abnormality detected.

Three hour OGTT was performed and showed

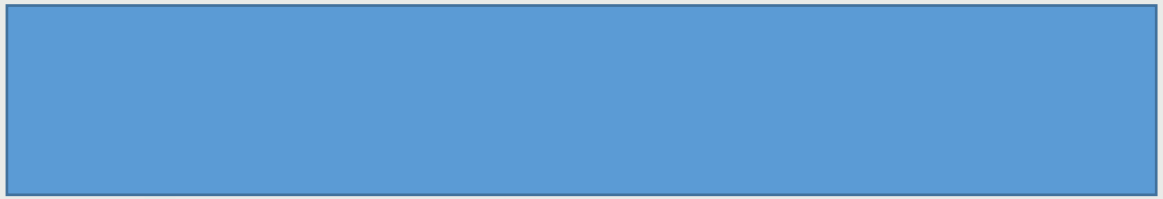
- Fasting plasma glucose- 105 mg/dl
- One hr plasma glucose- 190 mg/dl
- Two hr plasma glucose- 170 mg/dl
- Three hr plasma glucose- 155 mg/dl

Case 6

- 7 year old male child came with complaints of fatigue, increased frequency of urination, increased frequency of thrust, hunger.

- On Examination
  - Unintended weight loss
  - Increased irritability.

- Lab investigation
  - FBS- 350 mg/dl
  - Urine Examination
    - Urine appearance – Clear, pale
    - Urine sugar – 4 +
    - Urine ketone -++
    - 24 hour Urine volume – 2500 ml / day



Case 7

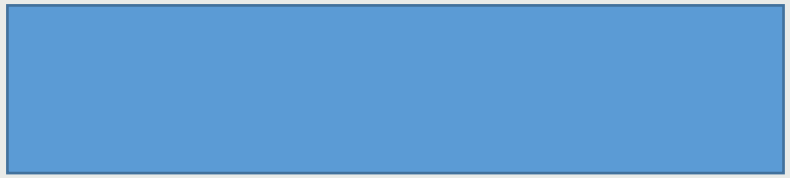
- 45 years old obese male patient came with complaints of weakness, increased frequency of thrust, hunger, increased frequency of urination.

- On Examination
  - Patchy dark velvety skin at armpits and neck.

- Lab investigation,
  - FBS- **180 mg/dl,**
  - PP – **380 mg/dl**
  - Urine :
    - Appearance – Clear pale
    - Fasting Urine sugar +
    - Post prandial Urine Sugar ++++
    - Urine Protein – Raised
    - Pus cells – 10-12 / hpf

Case 8

- 35 years old male came with complaints of increased frequency of urination at night.
- H/O Familial diabetes mellitus to father and elder brother.
- Lab investigation –
  - FBS – 124 mg/ dl,
  - PP Blood Sugar- 178 mg/dl
  - Urine – Pale yellow
  - Urine sugar – Nil
  - Urine protein – Nil



Case 9

- 24 years old female with 6 month of amenorrhea came with complaints of polyhydromnios, Pregnancy Induced Hypertension.
- h/o familial diabetes mellitus
- Lab Investigation –
  - FBS- 118 mg/dl
  - OGTT – 3 hour
    - Fasting – 118 mg/dl
    - Fasting plasma glucose-105 mg/dl
    - One hr plasma glucose- 190 mg/dl
    - Two hr plasma glucose- 170 mg/dl
    - Three hr plasma glucose- 155 mg/dl

Case 10

- 45 yrs male, presenting with c/o
  - Polyuria
  - Polydypsia
  - Weight loss

- Fasting blood glucose 150mg/dl
- Post prandial blood glucose 220mg/dl

Case 11

- 10 yr male,
- H/ o unexplained weight loss
- History of repeated infections at various sites
- Fasting BG 150 mg/DL
- Post prandial BG 340 mg/dl
- Serum insulin levels decreased (10 Miu/L)

Case 12

- 55 yr female
- Presented with nausea, vomiting, giddiness

- Laboratory Investigations:
  - Random BG 550mg/dl
  - Urine Sugar ++++
  - Urine ketone +++
  - HbA1c 13.5%

TABLE 33-3 Role of the Laboratory in Diabetes Mellitus

Diagnosis	
Preclinical (screening)	Immunological markers
	ICA
	IAA
	GAD antibodies
	Protein tyrosine phosphatase antibodies (IA-2)
	Zinc transporter ZnT8 antibodies
	Genetic markers (e.g., human leukocyte antigen [HLA])
	Insulin secretion
	Fasting
	Pulses
	In response to a glucose challenge
	Blood glucose
	Hemoglobin A1c (HbA_{1c})
Clinical	Blood glucose
	Oral glucose tolerance test (OGTT)
	HbA_{1c}
	Ketones (urine and blood)
	Other (e.g., insulin, C-peptide, stimulation tests)
Management	
Acute	Glucose
	Blood
	Urine
	Ketones
	Blood
	Urine
	Acid-base status (pH, bicarbonate)
	Lactate
	Other abnormalities related to cellular dehydration or therapy (e.g., potassium, sodium, phosphate, osmolality)
Chronic	Glucose
	Blood (fasting-random)
	Urine
	Glycated proteins
	Glycated hemoglobin (GHb) (HbA_{1c})
	Fructosamine
	Glycated serum albumin
	Urinary protein
	Urinary albumin excretion (UAE) (high albuminuria)
	Proteinuria
	Evaluation of complications (e.g., creatinine, cholesterol, triglycerides)
	Evaluation of pancreas transplant (C-peptide, insulin)
	Eligibility for insulin pump (C-peptide)