

Applied Blood Chemistry

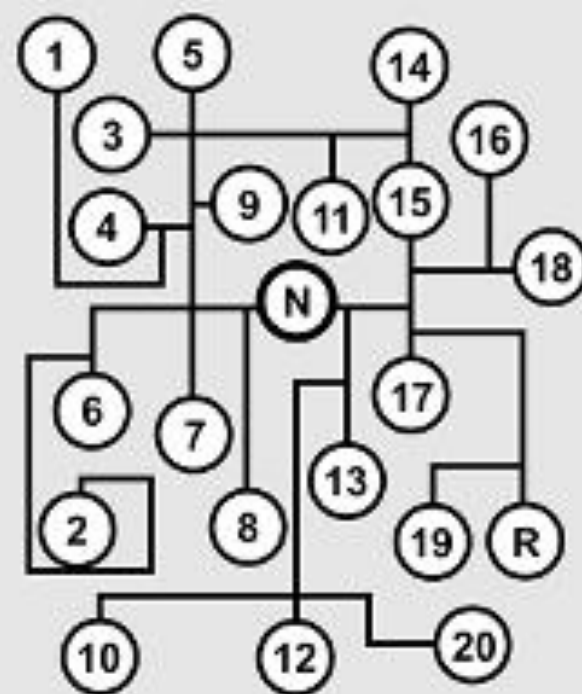
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Presented by Dr. BT Watts DC CSCS CFMP Pn1

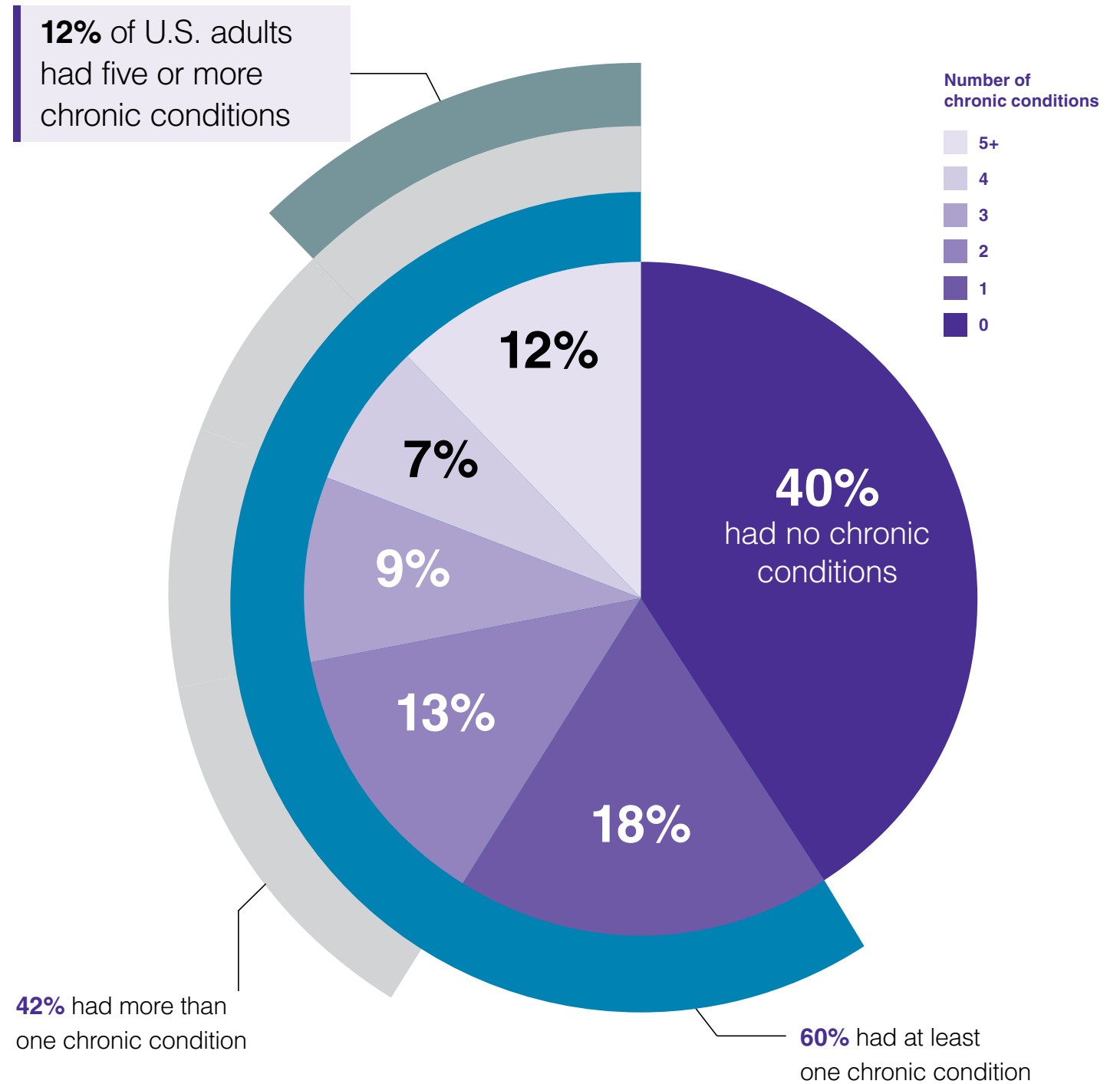
Disclaimer

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- * The information provided in this presentation is for your consideration only as a practicing health care provider. Ultimately you are responsible for exercising professional judgment in the care of your own patients.*

Shift Pattern

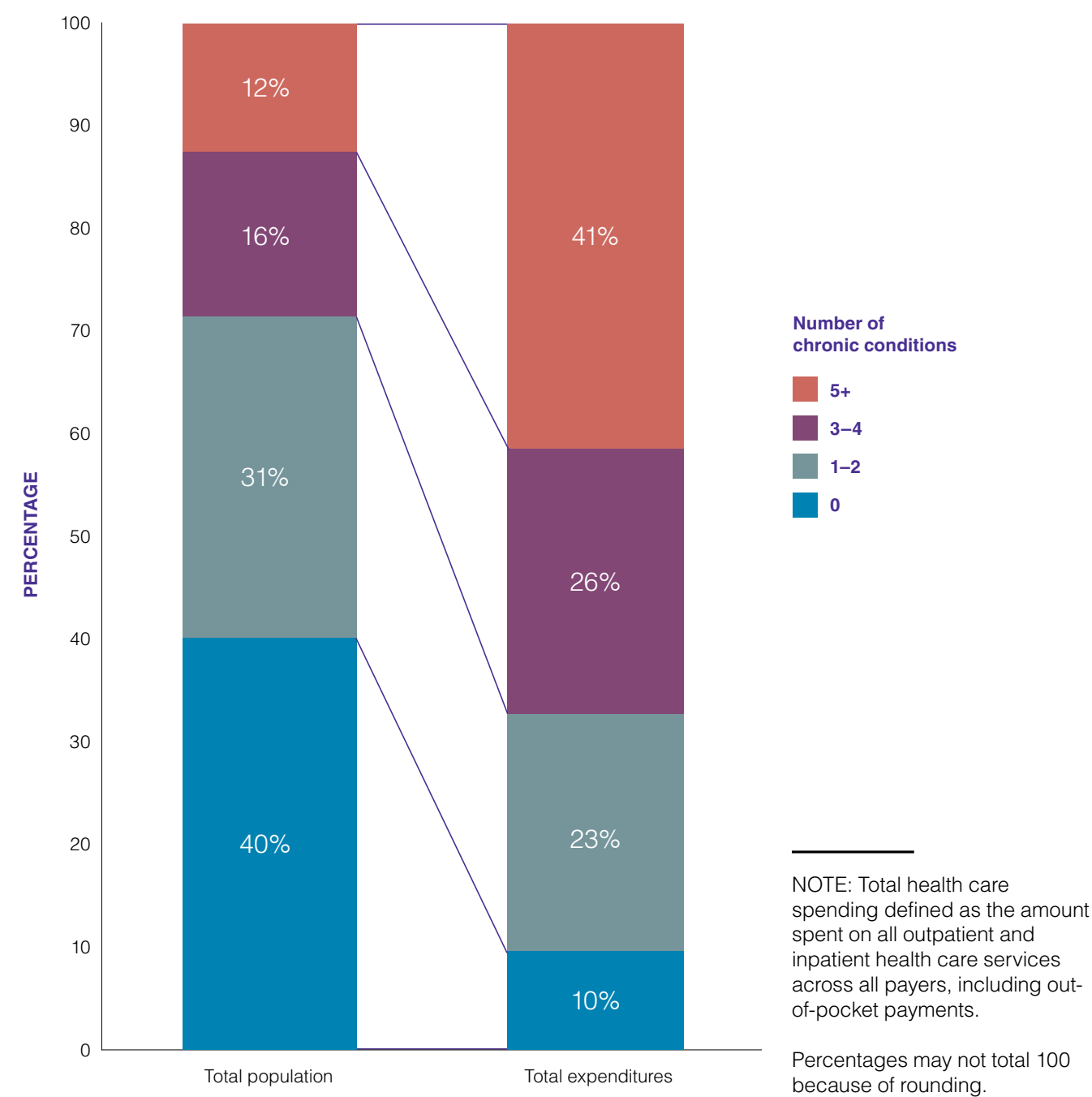


As of 2014, 60% of American adults had at least 1 chronic condition, 42% had more than 1 chronic condition.



NOTE: Percentages may not total 100 because of rounding.

Figure 2.2. Prevalence and Spending by Number of Chronic Conditions (2014)



Americans with 5 or more chronic conditions make up 12% of the population, but account for 41% of all health related expenses.

Patients with 5 or more chronic conditions spend 2x as much as those with 3-4, and **14x** as much as someone without any chronic condition at all.

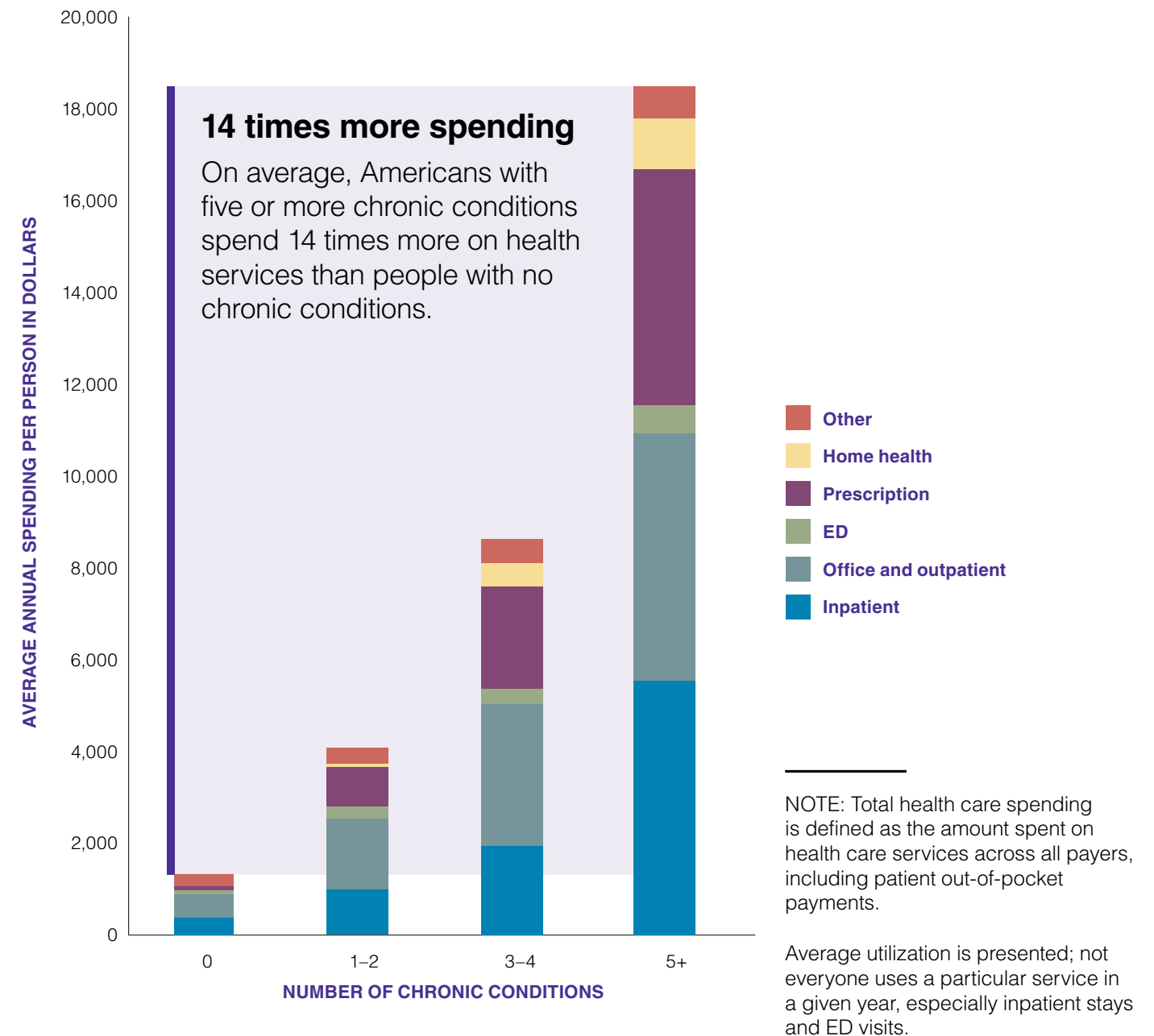
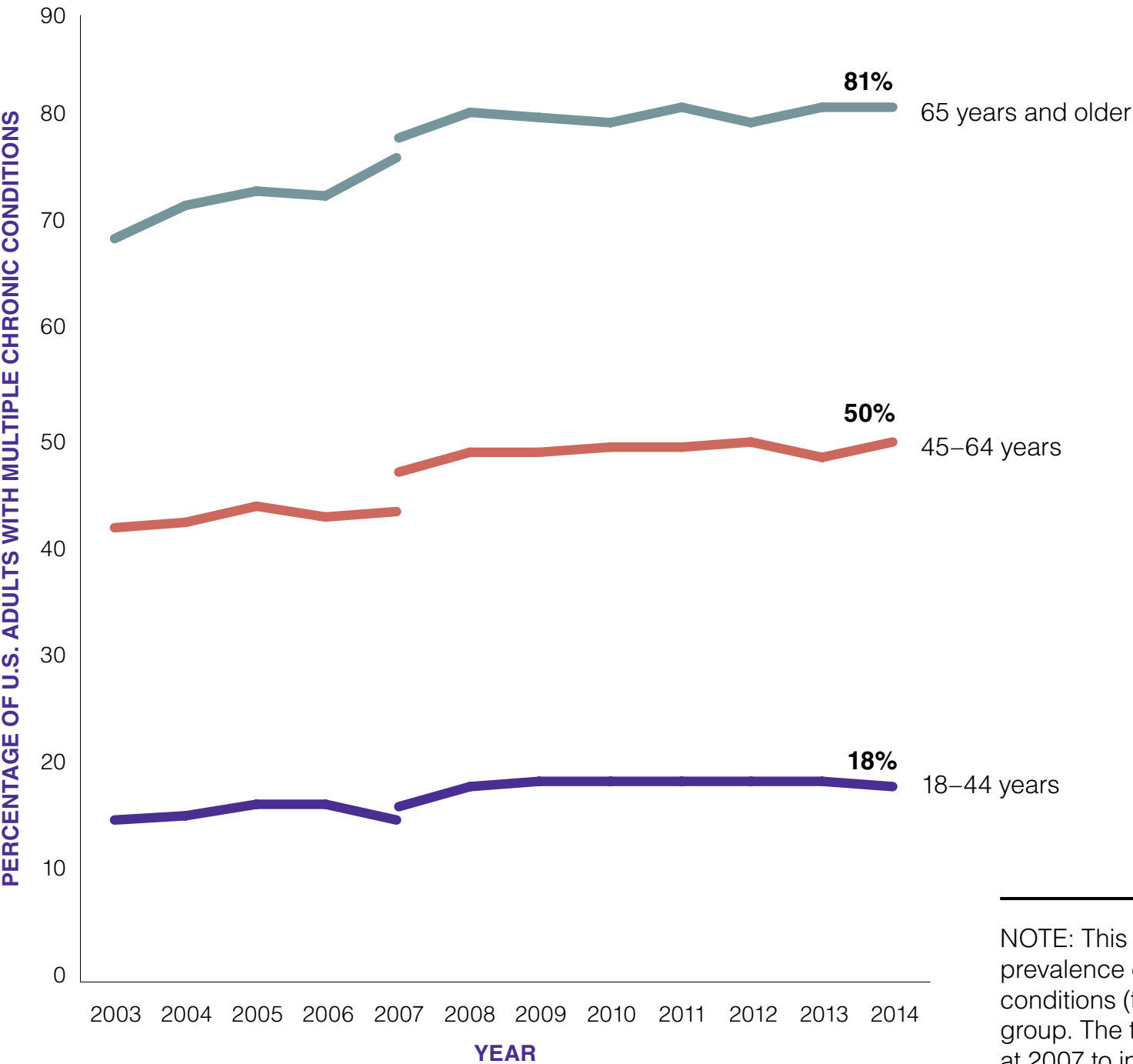


Figure A.1. Prevalence of Multiple Chronic Conditions (2003–2014)



NOTE: This figure documents the prevalence of multiple chronic conditions (two or more) by age group. The trend lines break at 2007 to indicate that MEPS changed its approach to the chronic disease questions.

Chronic Conditions and Lifespan

0 chronic conditions:	67+22.6 additional years
5 chronic conditions:	67+14.9 additional years
10 chronic conditions:	67+5 additional years



April 2018

Goldman Sachs asks in biotech research report: 'Is curing patients a sustainable business model?'

"...where the success of [its] hepatitis C franchise has gradually exhausted the available pool of treatable patients," the analyst wrote. "In the case of infectious diseases such as hepatitis C, curing existing patients also decreases the number of carriers able to transmit the virus to new patients, thus the incident pool also declines ... Where an incident pool remains stable (eg, in cancer) the potential for a cure poses less risk to the sustainability of a franchise."

People are not commodities...
unless THEY CHOOSE TO BE.

How do you transition your
approach?

re·spon·si·bil·i·ty

/rəˌspänsəˈbilədē/ 

noun

the state or fact of having a duty to deal with something or of having control over someone.

synonyms: authority, control, power, leadership

"a job with greater responsibility"

- the state or fact of being accountable or to blame for something.

"the group has claimed responsibility for a string of murders"

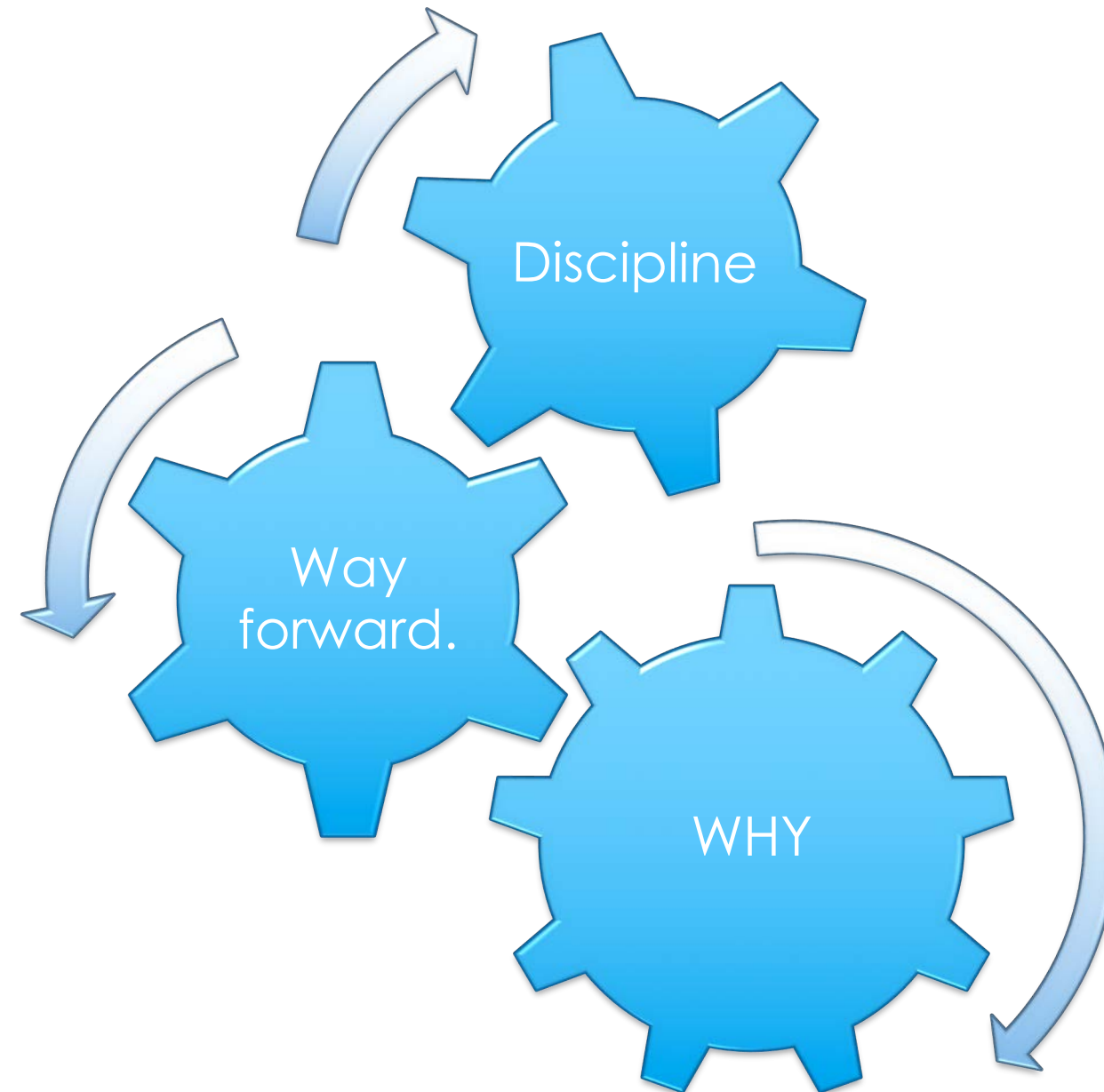
synonyms: blame, fault, guilt, culpability, liability

"they denied responsibility for the bomb attack"

- the opportunity or ability to act independently and make decisions without authorization.

"we would expect individuals lower down the organization to take on more responsibility"

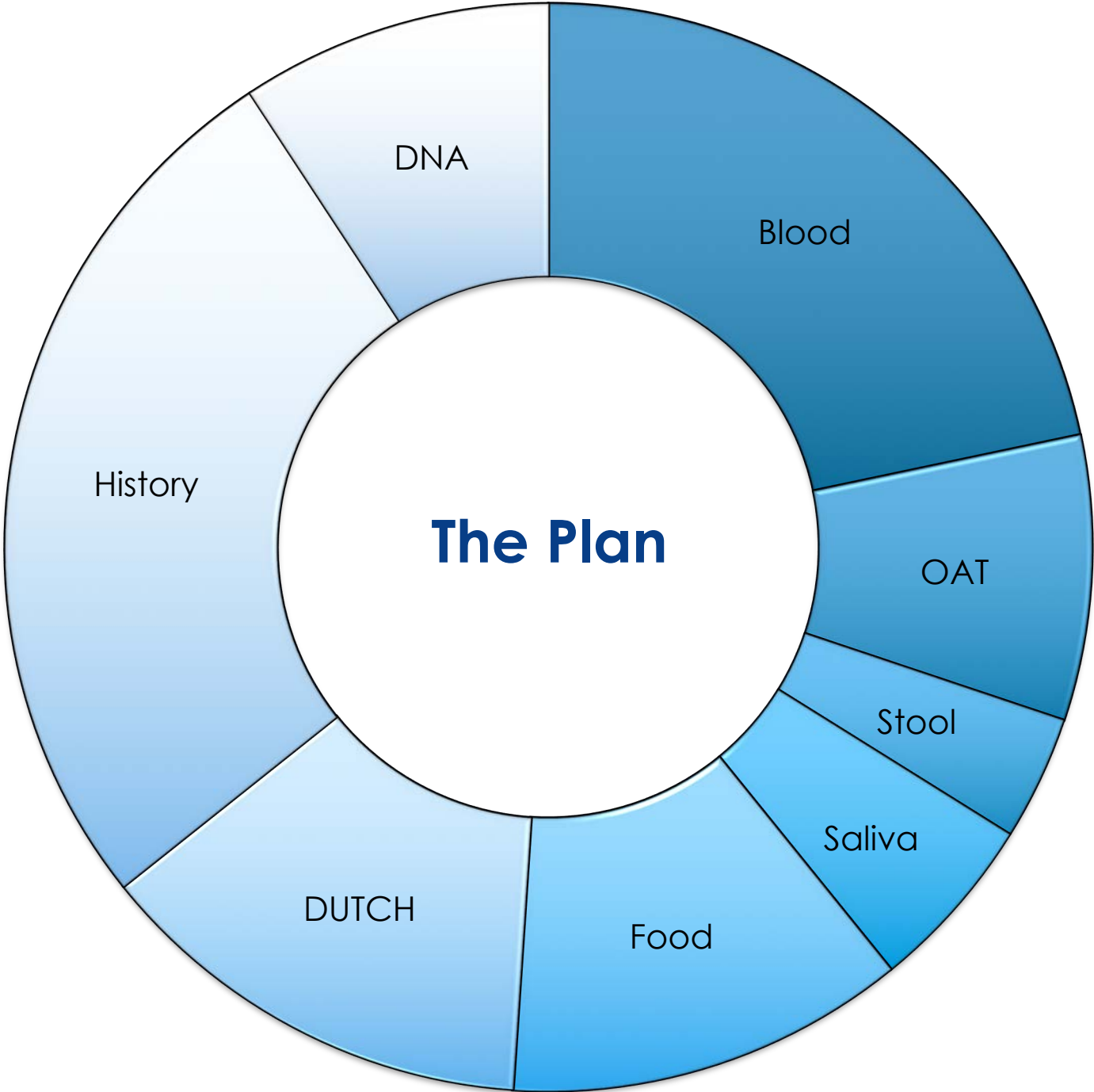
Work the Responsibility Machine



Responsibility Machine



Functional Medicine Diagnostic Workup



Applied FM



Responsibility Machine



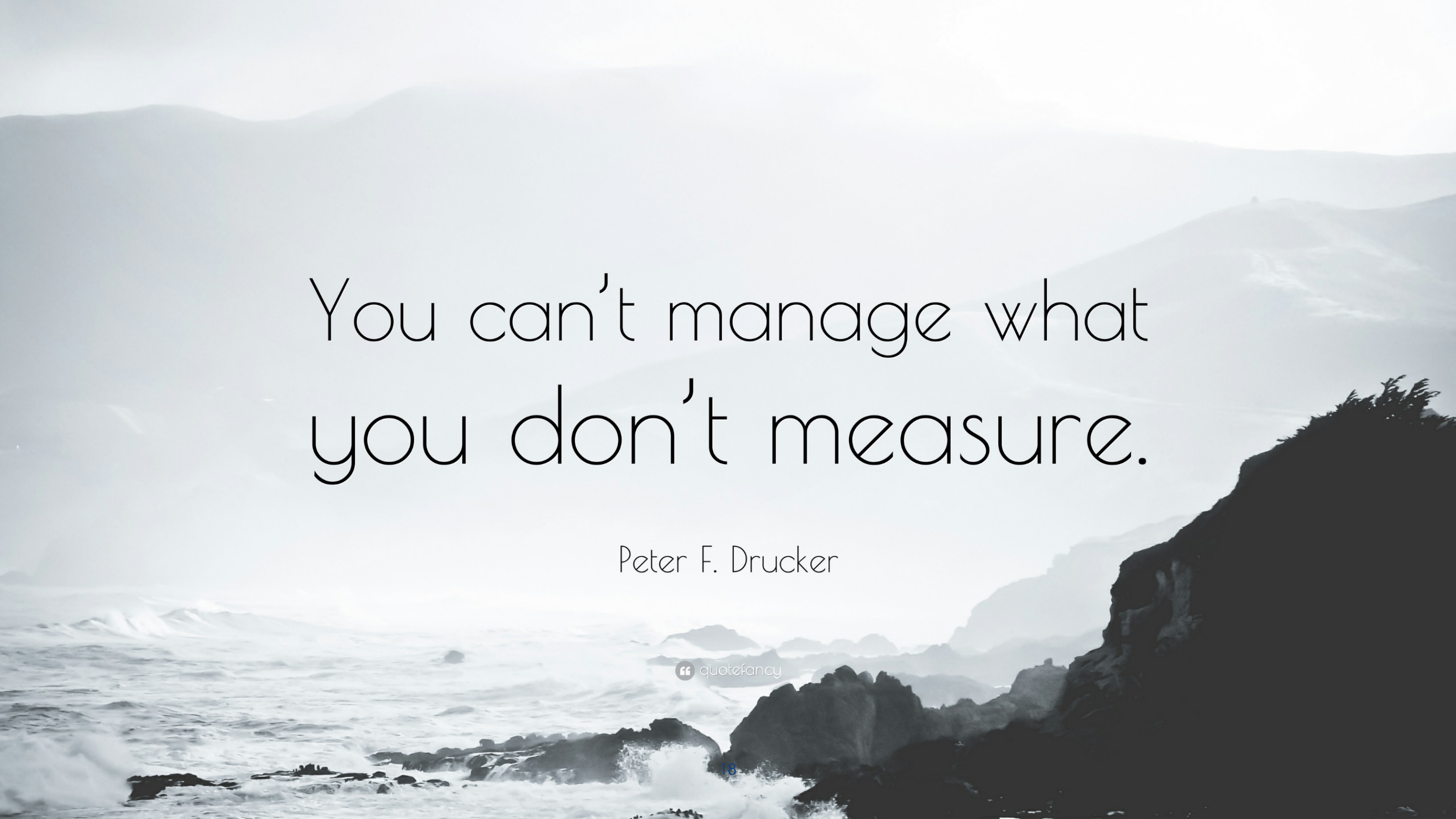
Functional Medicine Diagnostic Workup



Progress.

Why Blood Chemistry

1. Provides objective, measurable, reliable data.
2. Evaluates physiology for multiple body systems and processes including hormones, blood sugar regulation, kidney function, liver function, digestive function, cardiovascular health, oxygen carrying, oxidative stress, inflammation and more.
3. Scientifically validated test– it is the language used in health care worldwide

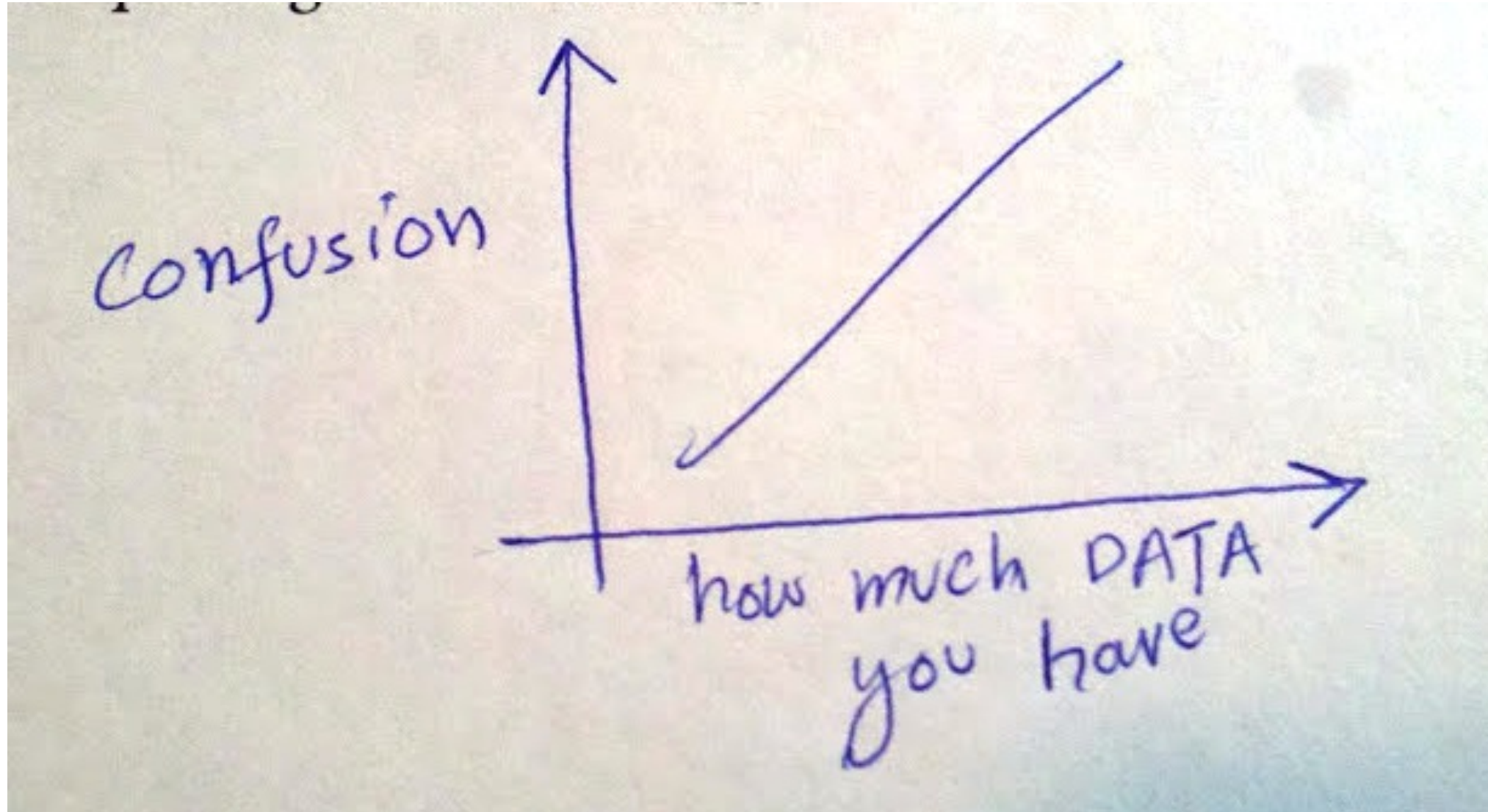


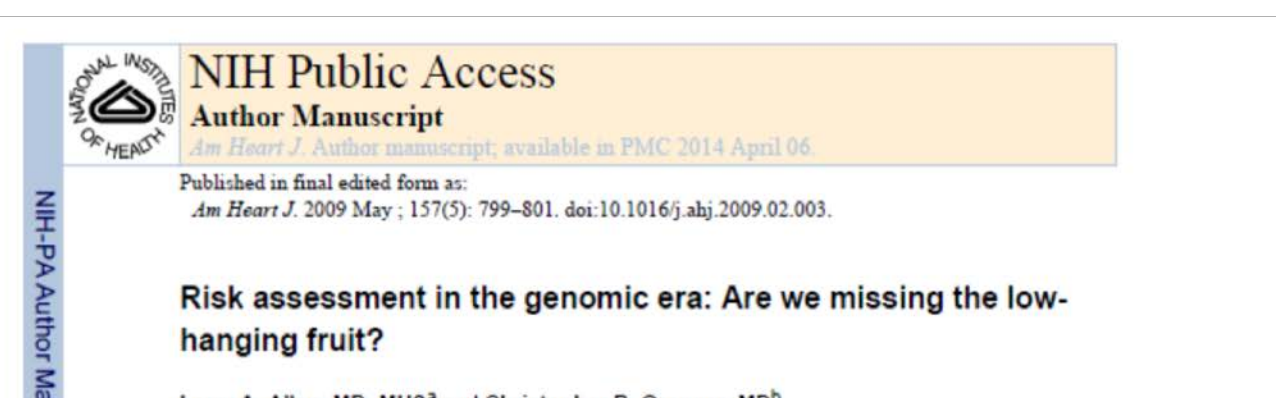
You can't manage what
you don't measure.

Peter F. Drucker

“ quote fancy

Too many labs?





At a minimum, there is a great deal of prognostic information in these standard laboratories that we are not currently using in a quantitative way. The authors should be commended for this ironic finding, that although enormous resources are being poured into identification of novel genomic risk markers, we have not adequately assessed what we already have at our fingertips every day.

The key message is that routinely available but less “novel” laboratory data must be considered. To go one step further, given the importance of efficiency in medical care, the default assumption should be to consider routine laboratory data of greater value than novel biomarkers *unless* the new techniques are shown by rigorous statistical testing to provide clinically significant incremental improvements in risk assessment or improve the coupling of risk prediction with therapeutic decision-making.

Product Name:	Codes
Bile Acids, Total	14801
Bilirubin, Direct	285
Cardio IQ® Lipoprotein Fractionation, Ion Mobility	91604
Cardio IQ® Lp-PLA2 Activity	94218
CBC (includes Differential and Platelets)	6399
Comprehensive Metabolic Panel (CMP-14)	10231
C-Peptide	372
Ferritin	457
Fibrinogen Activity, Clauss	461
Gamma Glutamyl Transferase (GGT)	482
GlycoMark®	19599
Hemoglobin A1c	496
Homocysteine	31789
hs-CRP	10124
Iron, Total and Total Iron Binding Capacity	7573
Lactate Dehydrogenase (LD)	593
Lipid Panel	7600
Magnesium	622
Phosphate (as Phosphorus)	718
Reticulocyte Count, Automated	793
Sedimentation Rate with Modified Westergren	809
T3 Uptake	861
T3, Free (FT3)	34429
T3, Total	859
T4 (Thyroxine), Total	867
T4, Free (FT4)	866
TSH (Thyroid Stimulating Hormone)	899
Uric Acid	905
Urinalysis, Complete	5463
Vitamin D, 25-Hydroxy, Total, Immunoassay	17306

The Way Forward

TODAY

1. Overview – composition of blood, basic concepts of blood chemistry testing, reference ranges
2. Glucose regulation (huge) and associated markers

TOMORROW

1. Anemia Panels
2. Electrolytes

A Few Things . . .

- * No such thing as a perfect lab test. They all have pros and cons.
- * Very few markers on a blood chemistry points to one thing (eg high TSH = hypothyroidism)
 - * Long list of possibilities for any given marker out of range

Current Findings :
1) Neutrophiles, High/Present
HEIRARCHICAL
1 Abscess pathophysiology
1 Abscess, lesser sac (567.2)
1 Bacterial infection (041.9 unspecified)
1 Epidural abscess (324.9)
1 Malignancy/cancer
1 Muscle abscess (728.89 unspecified)
1 Neoplasms/tumors
1 Periorbital cellulitis (376.01)
1 Right sided diverticulitis (562.00 unspecified)
1 Thigh cellulitis (682.6)
DRUGS
1 Corticosteroid administration
1 Fluphenazine (Prolixin) admin
1 Lithium (Lithane/Lithonate) admin
1 Lithium toxicity/overdose (985.8)
1 Niacin/Nicotinic acid admin
TRAUMA
1 Multiple trauma (959.8)
POISON AGENT SPECIFIC
1 Black widow spider bite (989.5 - E906.4)
1 Iron intoxication, acute (964.0)
1 Snakebite (rattlesnake/pit viper type) (989.5 - E906.2)
ELECTROMAGNETIC PHYSICS TRAUMA
1 Burns/severe/extensive (948.0 - 948.9)
1 Electrical injuries (994.8)
INFECTIOUS AGENT SPECIFIC
1 Amebiasis (006.9)
1 Bacterial tracheitis (464.10)
1 Eosinophilic meningoencephalitis (323.9)
1 Erysipelas (035)
1 Glanders (malleomyces mallei) (024)
1 Glanders abscess (024)
1 Lassa fever (078.89)
1 Legionaire's disease (482.84)
1 Leptospiral disease (100.9 leptospirosis, unspecified)
1 Leptospiral meningitis (100.81)
1 Leptospirosis/severe (Weils) type (100.0)
1 Meningococcemia/meningococcus (036.2 - 036.9)
1 Metastatic staphylococcus abscess synd (682.9)
1 Myocarditis, bacterial (422.92)
1 Primary bacterial peritonitis/ascites (567.2 - 789.5)
1 Rat bite fever, spirillary (026.0)
1 Respiratory syncytial virus infection (079.6)
1 Staphylococcus aureus infection (041.11)

1 Streptococcal pharyngitis, beta type A (034.0)
1 Tetanus
1 Trichinosis (124)
1 Tularemia (021.9)
INFECTED ORGAN ABSCESS
1 Abscess Bartholin gland cyst (616.2)
1 Abscess, abdominal, visceral (567.2)
1 Abscess, cervical gland (682.1)
1 Abscess, dentoalveolar (522.5)
1 Abscess, gallbladder/pericholecystic (575.0)
1 Abscess, intracranial (324.0)
1 Abscess, intraperitoneal, RL quadrant (567.2)
1 Abscess, ischiorectal (566)
1 Abscess, liver (572.0)
1 Abscess, myocardium (422.92)
1 Abscess, parapharyngeal (478.29)
1 Abscess, paraspinal (324.1)
1 Abscess, perianal/pararectal (566)
1 Abscess, periapical (522.5)
1 Abscess, renal (590.2)
1 Abscess, retroperitoneal (567.2)
1 Abscess, retropharyngeal (478.24)
1 Abscess, subarachnoid (324.9)
1 Abscess, subcutaneous (682.9 unspecified)
1 Abscess, submammary (611.0)
1 Abscess, submastoid/Bezold's abscess (383.01)
1 Abscess, subphrenic (998.59)
1 Anterior tibial compartment/fasciitis (729.4 unspecified)
1 Appendicitis (541)
1 Appendix abscess (540.1)
1 Areolar abscess/nipple (661.0)
1 Arthritis (716.90)
1 Bacterial endocarditis/acute (421.0)
1 Brain abscess (324.0)
1 Breast abscess (611.0)
1 Brodie's abscess (730.1)
1 Cellulitis (682.9)
1 Cellulitis, chronic pelvic (614.4)
1 Cellulitis, dissecting, of scalp (704.8)
1 Cellulitis, pelvic, acute (614.3)
1 Cellulitis, periurethral, male (597.0)
1 Cellulitis/lower extremities (682.6 - 682.7)
1 Cellulitis/pinna (380.10)
1 Cerebral abscess/congen. cardiac anomly (324.0)
1 Cervical adenitis/abscess (289.5 - 289.0)
1 Cholangitis, ascending (576.1)
1 Cholecystitis without gallstones (575.0)
1 Cholecystitis, acute (575.0)
1 Divericulitis/phlegmon/abscess (562.11 - 682.9)
1 Emphysematous cholecystitis (575.0)
1 Endocarditis, infective (421.0)
1 Endometritis, acute (615.0)
1 Gingiva, abscess (523.3)
1 Hepatic abscess, pyogenic (572.0)
1 Intraperitoneal abscess (567.2 - 567.0)
1 Lacrimal gland infection/abscess (375.03)
1 Lung abscess (513.0)

1 Mastoiditis, zygomatic (383.9 unspecified)
1 Meckel's acute diverticulitis (751.0)
1 Mediastinal abscess (513.1)
1 Nasal septum abscess/hematoma (920)
1 Omentum abscess (567.2)
1 Osteomyelitis (730.2)
1 Osteomyelitis, vertebral (730.28)
1 Ovarian abscess/Tubo-ovarian abscess (614.2)
1 Pancreas abscess (577.0)
1 Pelvic abscess (614.4)
1 Pelvic inflammatory disease (614.9)
1 Penis cellulitis (607.2)
1 Perinephric abscess (590.2)
1 Peritonitis, acute (567.9)
1 Peritonsillar abscess (475)
1 Periurethral abscess (597.0)
1 Prostate abscess (601.2 - 098.12)
1 Prostatitis, acute (601.0)
1 Psoas abscess (728.89)
1 Puerperal pelvic cellulitis/sepsis (670.00)
1 Pyopneumothorax (510.9 - 510.0)
1 Scrotum abscess (608.4)
1 Septicemia (038.9)
1 Sialadenitis (527.2)
1 Spinal epidural abscess (324.1)
1 Splenic abscess (289.59)
1 Stomach abscess (535.0)
1 Subhepatic/gallbladder bed abscess (575.0)
1 Submaxillary gland infection/abscess (527.3)
1 Suppurative lymphangitis (682.9)
1 Testis, abscess (604.0)
1 Tibia osteomyelitis (015.7)
1 Tonsil, abscess (475)
1 Vitreous, abscess (360.04)
GRANULOMATOUS INFLAMMATORY
1 Pancreatitis, acute (577.0)
METABOLIC STORAGE DISORDER
1 Diabetic ketoacidosis/coma (250.3)
ALLERGIC COLLAGEN AUTO-IMMUNE
1 Anaphylaxis, generalized (995.0)
1 Dressler's syndrome (411.0)
1 Hypersensitivity pneumonitis (495.9)
1 Nephrotic syndrome (581.9)
1 Polyarteritis nodosa (446.0)
1 Purpura fulminans (286.6)
1 Reiter's syndrome (099.3)
1 Rheumatic fever (390)
1 Serum sickness (999.5 unspecified)
1 Temporal/cranial arteritis (446.5)
1 Thrombotic thrombocytopenic purpura (287.3)
1 Urticaria (708.9)
CONGENITAL DEVELOPMENTAL

1 Granulomatous dis/childhood, chronic (686.1)
1 Hemolytic disease of newborn (773.2)
HEREDITOFAMILIAL
1 Agranulocytosis, infantile hereditary (288.0)
1 Sickle cell anemia (282.60)
1 Sickle cell crisis (282.62)
RELATIONAL PSYCHIATRIC
1 Stress/Emotional/Physical (308.0)
STRUCTURAL ANATOMIC FOREIGN BODY
1 Acute gastric acid/aspiration syndrome (536.0 - 535.40)
1 Choledocholithiasis (574.50)
1 Gastroduodenal perforation (533.90 - 533.91)
1 Pulmonary embolism (415.11)
1 Thrombophlebitis, peripheral (451.2)
1 Thrombosis, arterial (444)
ARTERIOSCLEROTIC VASCULAR
1 Cerebral hemorrhage (431)
1 Gangrene, ischemic (785.4 - 440.24)
1 Infarction, bowel (557.0)
1 Myocardial infarction, acute (410.90)
1 Pulmonary infarction (415.10)
FUNCTIONAL PHYSIOLOGIC
1 Exercise/Aerobic exercise (V57.0)
VEGETATIVE AUTONOMIC
1 Cushing's disease (255.0)
1 Eclampsia of pregnancy (642.60)
1 Mastitis, puerperal (675.20)
REFERENCE TO ORGAN SYSTEM
1 Hemorrhage, acute (459.0)
1 Leukemoid reaction (288.8)
1 Renal failure, acute (586)
NEOPLASTIC
1 Adenocarcinoma, pancreatic (157.9)
1 Carcinoma, bronchogenic (162.9 primary - 212.3 benign)
1 Carcinoma, hepatocellular (155.0)
1 Hodgkins disease (201.90)
1 Metastatic malignancy (cancer)
1 Myelogenous leukemia, chronic (205.1)
1 Myelogenous/Blastic Leukemia, Acute (205.00)
1 Myeloproliferative disease (238.7)
1 Polycythemia vera (238.4)

HEIRARCHICAL
1 Aflatoxicosis/Aflatoxin ingest/effect (989.7) 1 Heart disorders (427.9) 1 Liver disorders (573.9) 1 Muscle disorders (728.9) 1 Obstructive jaundice syndrome (576.8)
DRUGS
1 Cholestasis, estrogen/OC pill induced (576.8 cholestasis) 1 Drug-induced hepatitis (573.3) 1 Isoniazid hepatitis (573.3)
TRAUMA
1 Crush syndrome (muscle injury severe) (958.5) 1 Heat stroke (992.0) 1 Myocardial contusion (861.01)
POISON AGENT SPECIFIC
1 Alcohol intoxication, acute (305.00) 1 Alcoholic liver disease (571.3 unspecified) 1 Alcoholism, chronic (303.90 unspecified) 1 Drug abuse/recreational/substance abuse (305.9) 1 Haff disease poisoning (985.1) 1 Halothane exposure liver disease (968.1) 1 Mushroom poisoning/all types (988.1)
POISONED ORGAN
1 Alcoholic fatty liver (571.0) 1 Cirrhosis, portal (Laennec's alcoholic) (571.2) 1 Hepatitis, alcoholic (571.1) 1 Hepatitis, toxic (573.3) 1 Neuroleptic malignant syndrome (333.92)
ELECTROMAGNETIC PHYSICS TRAUMA
1 Hypothermia, accidental/exposure (991.6)
INFECTIOUS AGENT SPECIFIC
1 Alveolar hydatid disease (122.9) 1 Amebic abscess, liver (006.3) 1 Anicteric viral hepatitis (070.9) 1 Brucellosis (023.9) 1 Chronic malaria (084.6) 1 Cirrhosis, syphilitic (Hepar lobatum) (095.3) 1 Cytomegalic infection, disseminated (078.5) 1 Cytomegalic mononucleosis syndrome (078.5) 1 Delta agent hepatitis (070.33) 1 Ehrlichiosis (082.40) 1 Hepatic brucellosis (023.9) 1 Hepatic leptospirosis (100.0) 1 Hepatitis A (070.1) 1 Hepatitis B (070.30) 1 Hepatitis B vasculitis syndrome (447.6 vasculitis - 070.30 Hep

B)
1 Hepatitis C virus (non-A, non-B) (070.51) 1 Hepatitis E virus (070.53) 1 Hepatitis G virus (573.3 unspecified) 1 Hepatitis, amebic (006.3) 1 Hepatitis, cholestatic, viral type (573.8) 1 Hepatitis, chronic active/HBV (571.49) 1 Hepatitis, chronic C type (070.54) 1 Hepatitis, complicating (non-hep virus) (573.3) 1 Hepatitis, mononucleosis (075) 1 Hepatitis, persistent, chronic (571.41) 1 Hepatitis, toxoplasmosis (130.5) 1 Hepatitis, viral (070.9) 1 Hepatitis/chronic aggressive (571.49) 1 Infectious mononucleosis (075) 1 Kawasaki disease (446.1) 1 Legionaire's disease (482.84) 1 Leptospiral disease (100.9 leptospirosis, unspecified) 1 Leptospirosis/severe (Weils) type (100.0) 1 Listeria hepatitis, newborn (774.4 - 027.0) 1 Perihepatitis, gonococcal (098.86) 1 Plasmodium falciparum malaria (084.0) 1 Q fever (083.0) 1 Reye's syndrome (331.81) 1 Rubella (056.9) 1 Scrub typhus (081.2) 1 Toxic shock syndrome (040.89) 1 Typhoid fever (002.0) 1 Typhus, acute/epidemic (080)
INFECTED ORGAN ABSCESS
1 Cholecystitis without gallstones (575.0) 1 Cholecystitis, acute (575.0) 1 Drug-induced Hepatitis, idiosyncratic (573.3) 1 Hepatitis, bacterial (070.1) 1 Hepatitis, granulomatous (573.3 - 681.1 granuloma)
GRANULOMATOUS INFLAMMATORY
1 Pancreatitis, acute (577.0) 1 Sarcoidosis (135)
METABOLIC STORAGE DISORDER
1 Cryoglobulinemia, idiopathic (273.2 - 273.3) 1 Diabetic ketoacidosis/coma (250.3) 1 Erythrohepatic protoporphyria (277.1) 1 Fructose intolerance, hereditary (271.2) 1 Hemochromatosis (275.0) 1 LCAT deficiency 1 Wilsons Disease (275.1)
ALLERGIC COLLAGEN AUTO-IMMUNE
1 Autoimmune hepatitis (Plasma cell) (571.49) 1 Cirrhosis, primary biliary (571.6) 1 Dermatomyositis (710.3) 1 Polymyositis (710.4)

1 Primary sclerosing cholangitis (571.6) 1 Transplanted liver rejection (996.82) 1 Ulcerative colitis (556.9)
CONGENITAL DEVELOPMENTAL
1 Cirrhosis (571.5) 1 Cirrhosis, congenital/neonatal (777.8)
USE DEGENERATIVE NECROSIS AGE
1 Hepatic necrosis, acute/subacute (570) 1 Rhabdomyolysis (728.89)
ARTERIOSCLEROTIC VASCULAR
1 Hepatic vein occlusion/thrombus (453.0) 1 Myocardial infarction, acute (410.90) 1 Veno-occlusive hepatic disease (459.9)
VEGETATIVE AUTONOMIC
1 Disseminated intravascular coagulation (286.6) 1 Eclampsia of pregnancy (642.60) 1 Hypothyroidism (myxedema) (244.9) 1 Myxedema coma (244.9) 1 Thyrotoxic crisis (242.90)
REFERENCE TO ORGAN SYSTEM
1 Chronic liver disease (571.9) 1 Cirrhosis, cardiac (571.5) 1 Cirrhosis, postnecrotic (571.5) 1 Cryoimmunoglobulinemic syndrome (289.9) 1 Fatty liver (571.8) 1 Fatty liver of pregnancy, acute (571.8) 1 Fulminant hepatic failure (570) 1 HELLP syndrome of pregnancy (V22.1) 1 Hepatic encephalopathy (572.2) 1 Hepatic failure (572.8) 1 Hepatic function impairment (573.8) 1 Jaundice of pregnancy, recurrent (782.4) 1 Peliosis hepaticus (573.9) 1 Right congestive heart failure/chronic (428.0) 1 Steatosis/Non-alcoholic fatty liver dis (571.8)
NEOPLASTIC
1 Adenocarcinoma, gastric (151.9) 1 Angiosarcoma (171.9) 1 Carcinoma, gallbladder (156.0) 1 Carcinoma, hepatocellular (155.0) 1 Cholangiocarcinoma, intrahepatic (155.0) 1 Lymphoblastic leukemia, acute (208.00) 1 Lymphoma/malignant, non-Hodgkins (201.9) 1 Metastatic liver disease (197.7)

A Few Things . . .

- * No such thing as a perfect lab test. They all have pros and cons.
- * Very few markers on a blood chemistry points to one thing (eg high TSH = hypothyroidism)
 - * Long list of *possibilities* for any given marker out of range
 - * You can narrow down any given abnormality to a shorter list of *probabilities*
- * Then make some changes, and in a few months re-run the blood chemistry

Basic Laboratory Panels

- * Chemistry (eg chem-7, chem-14)
 - * Enzymes, electrolytes, wastes, proteins, etc.
- * CBC with differential
 - * Red blood cells and their indices, white blood cells, platelets
- * Lipids
 - * Cholesterol, HDL, LDL, triglycerides
- * Thyroid panel
 - * Thyroid function

Functional Panels

Liver (hepatic) function

- * Albumin, AST, ALT, GGT, alkaline phosphatase, bilirubin, protein, globulin

Kidney (renal) function

- * BUN, creatinine, phosphorus, calcium, glucose, chloride, CO₂

Electrolyte, hydration and pH

- * Sodium, potassium, magnesium, chloride, CO₂, calcium, phosphorus

Cardiovascular panel

- * Total cholesterol, HDL, LDL, triglycerides
- * Advanced markers

CBC with differential

- * Red blood cell count
- * Hemoglobin
- * Hematocrit
- * MCV
- * MCH
- * MCHC
- * RDW
- White blood cell count
- Neutrophils
- Lymphocytes
- Monocytes
- Eosinophils
- Basophils
- Platelet count

Lipid Panel

- * Total Cholesterol
- * LDL cholesterol
- * HDL cholesterol
- * Triglycerides

Metabolic 14

- * Glucose
- * Sodium
- * Potassium
- * Chloride
- * Carbon dioxide (bicarbonate)
- * Calcium
- BUN (blood urea nitrogen)
- Creatinine
- Bilirubin
- AST, ALT, alk phos
- Albumin
- Globulin

Also included

- * GGT
- * Uric acid
- * Phosphorus
- * LDH
- * Iron
- * Magnesium
- * 25-Vitamin D
- * Ferritin
- * Bile acids

Thyroid Panel

- * TSH
- * Total T4 (thyroxine)
- * Total T3 (triiodothyronine)
- * Free T4
- * Free T3
- * T3 uptake

Cardio Panel

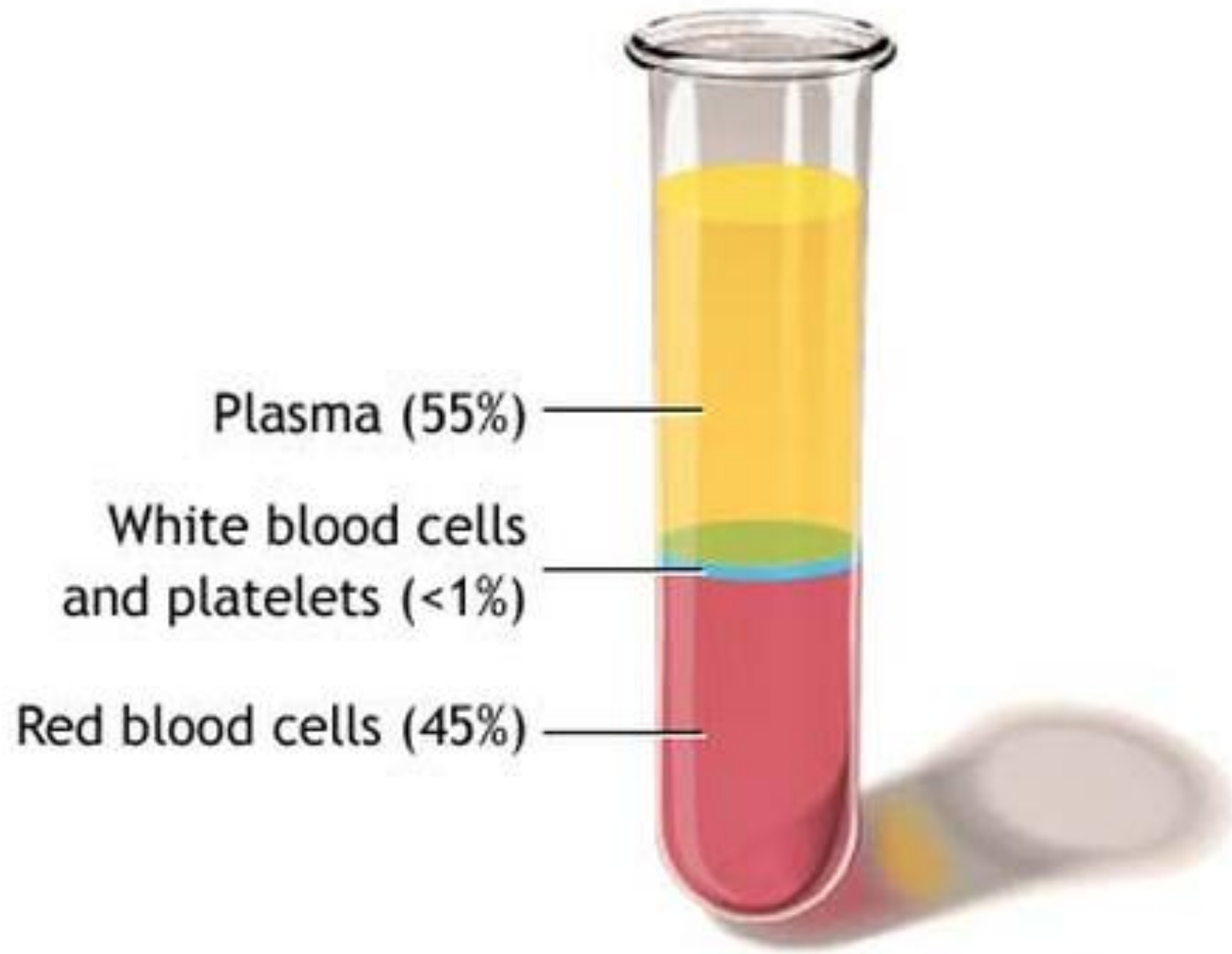
- * Cardio IQ Lipoprotein Fraction
- * Cardio IQ Lp-PLA2 activity
- * Fibrinogen
- * Homocysteine
- * hs-CRP
- * ESR

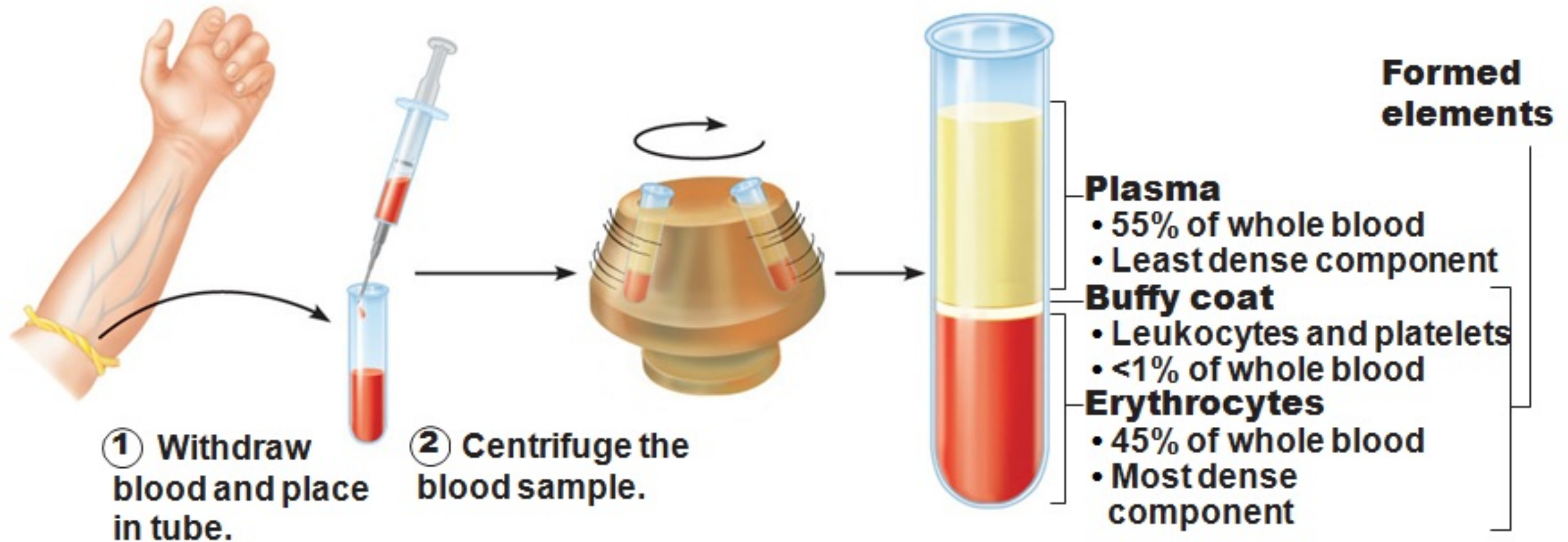
Glucose Regulation

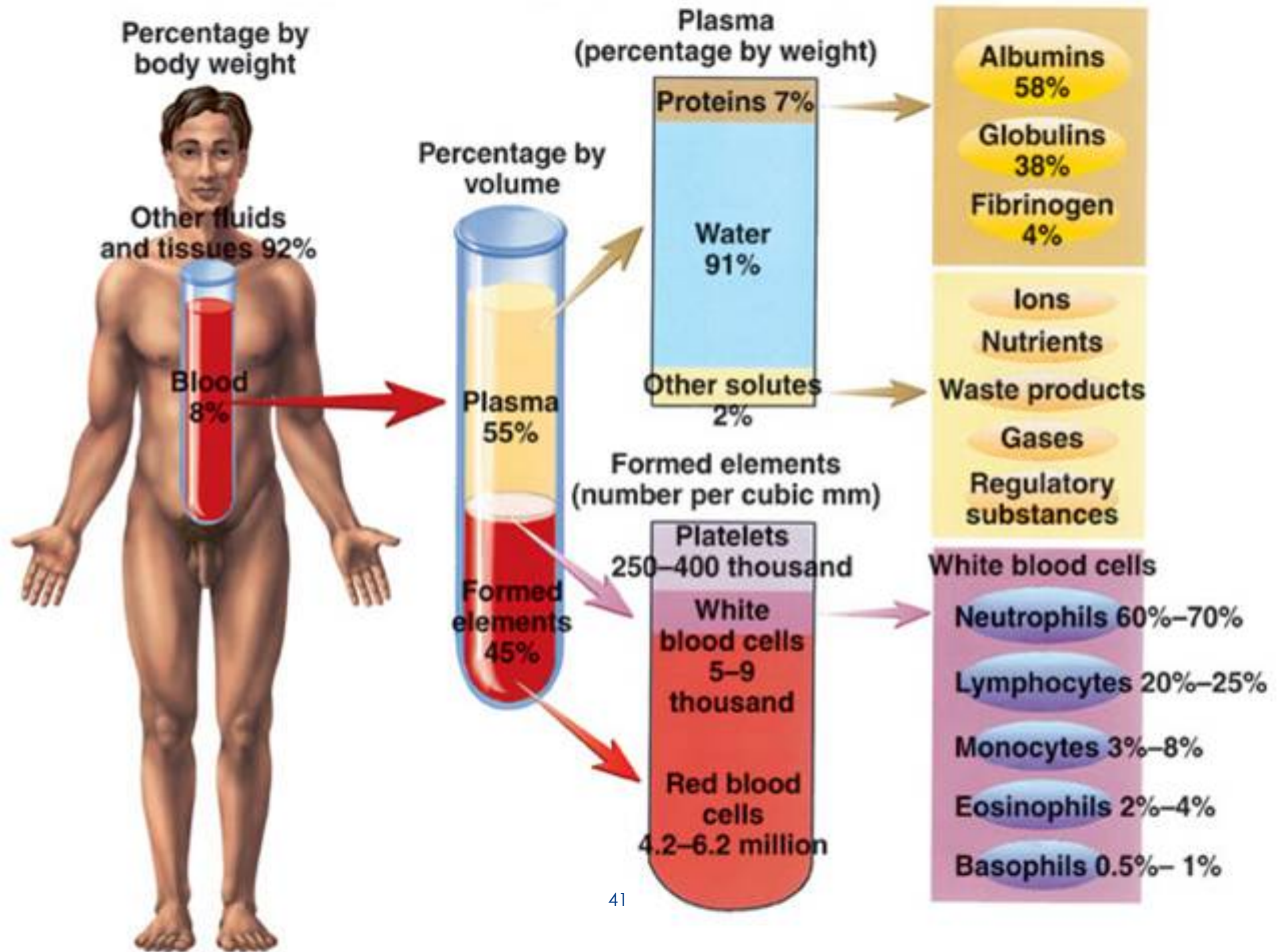
- * Hemoglobin A1C
- * GlycoMark
- * C-peptide

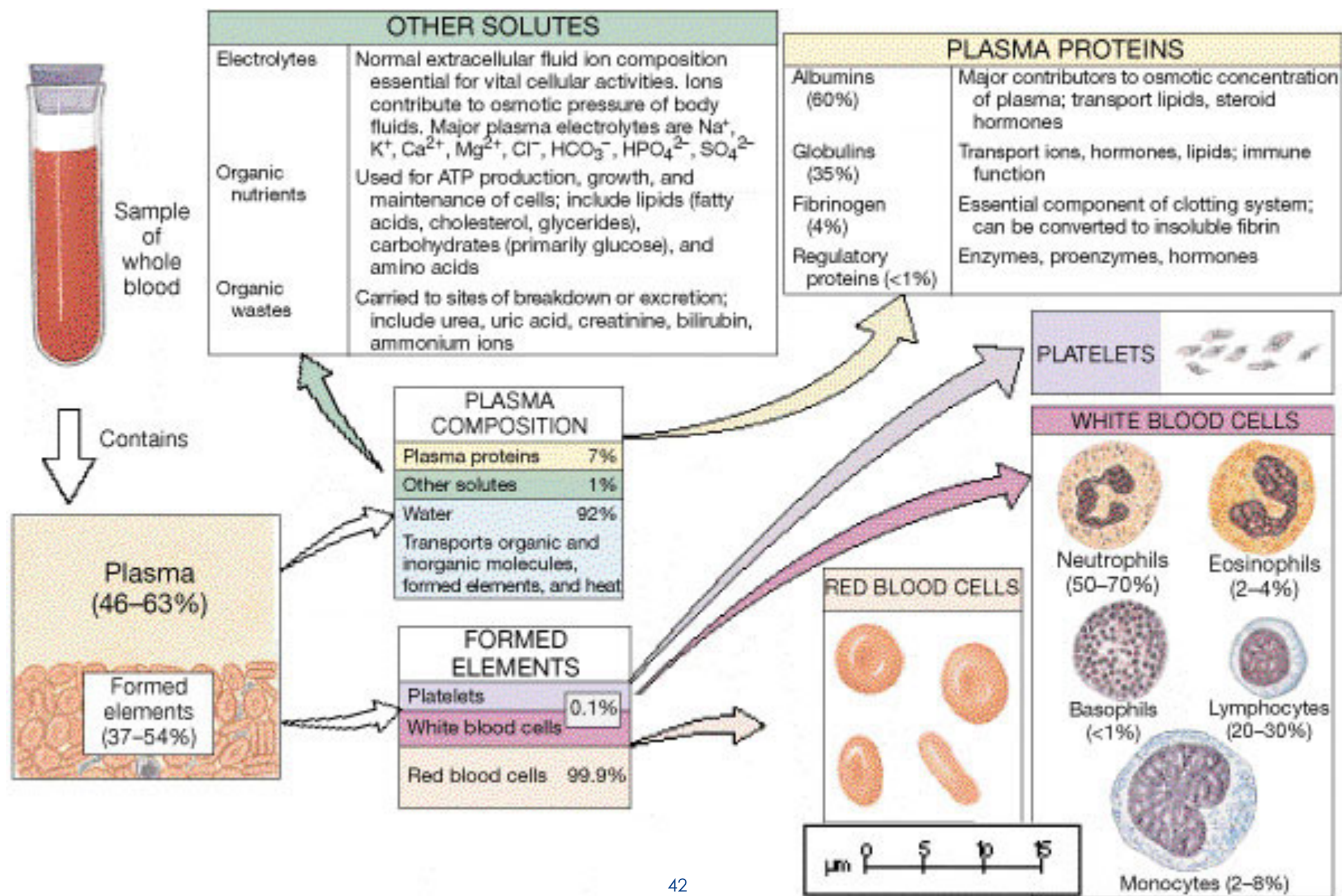
Section Break Number 1

What Is Blood?

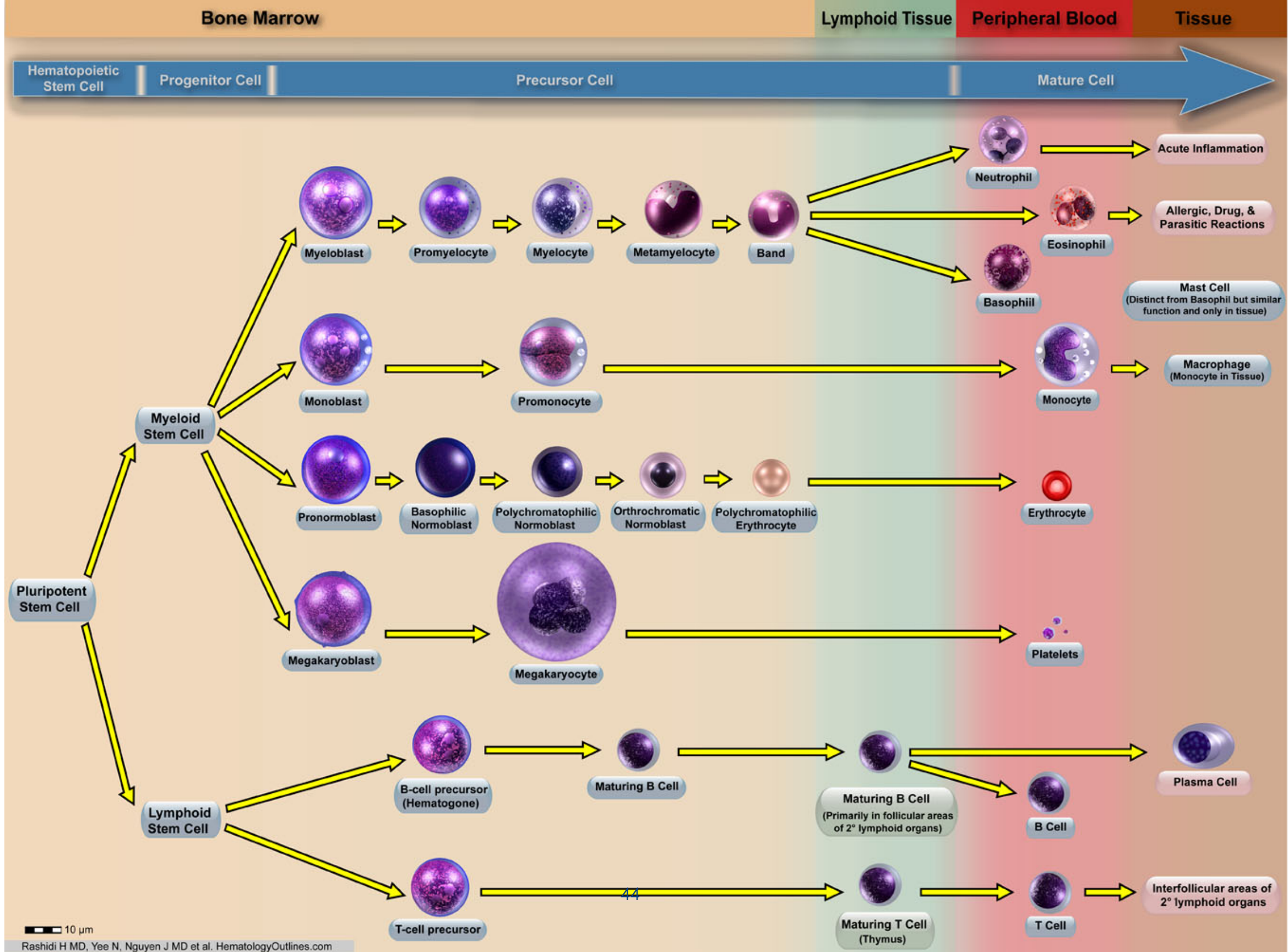




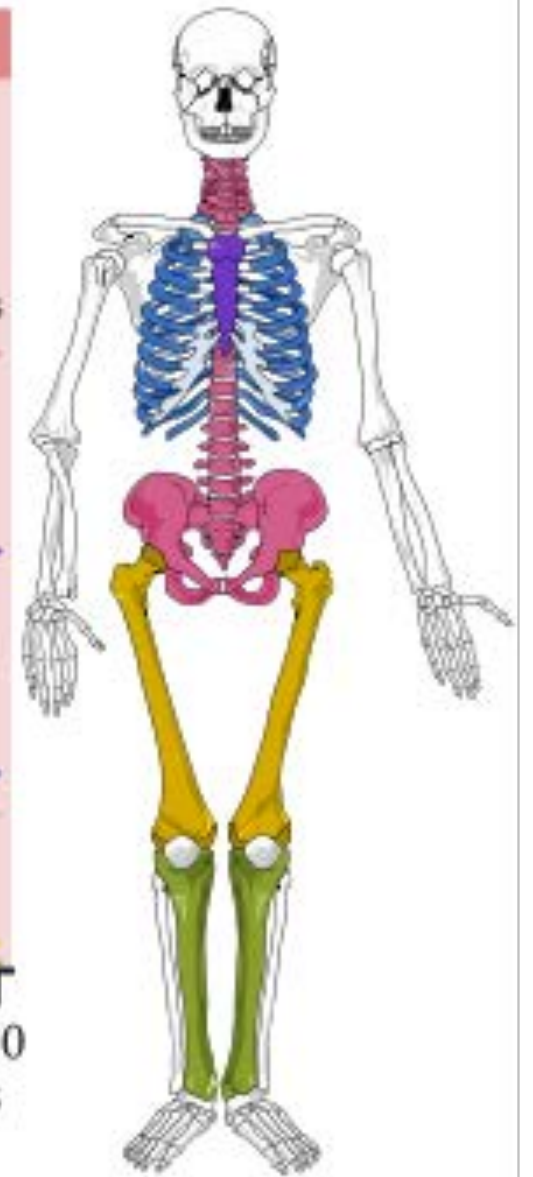
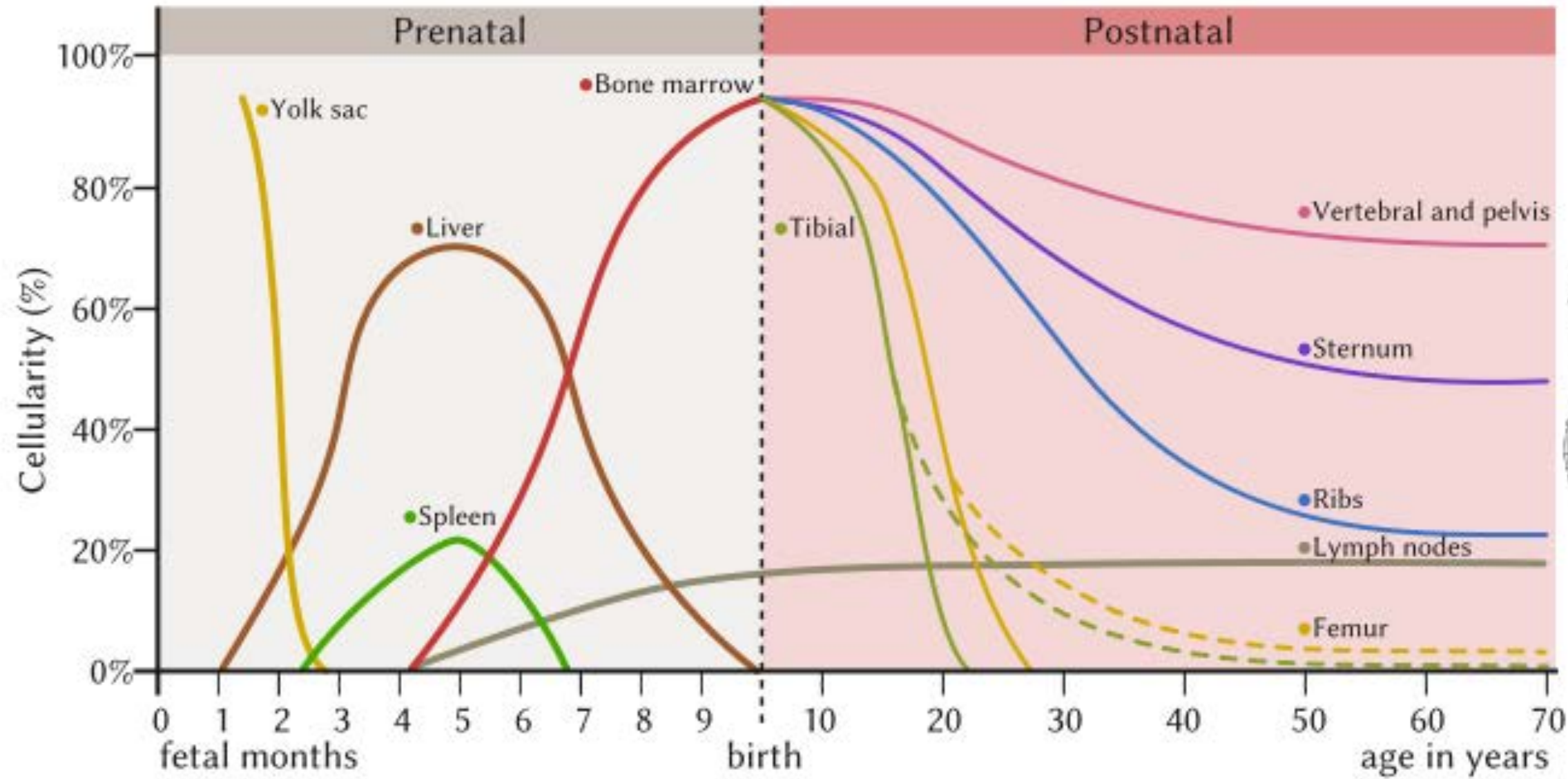




Where is blood made?



HEMATOPOIESIS•



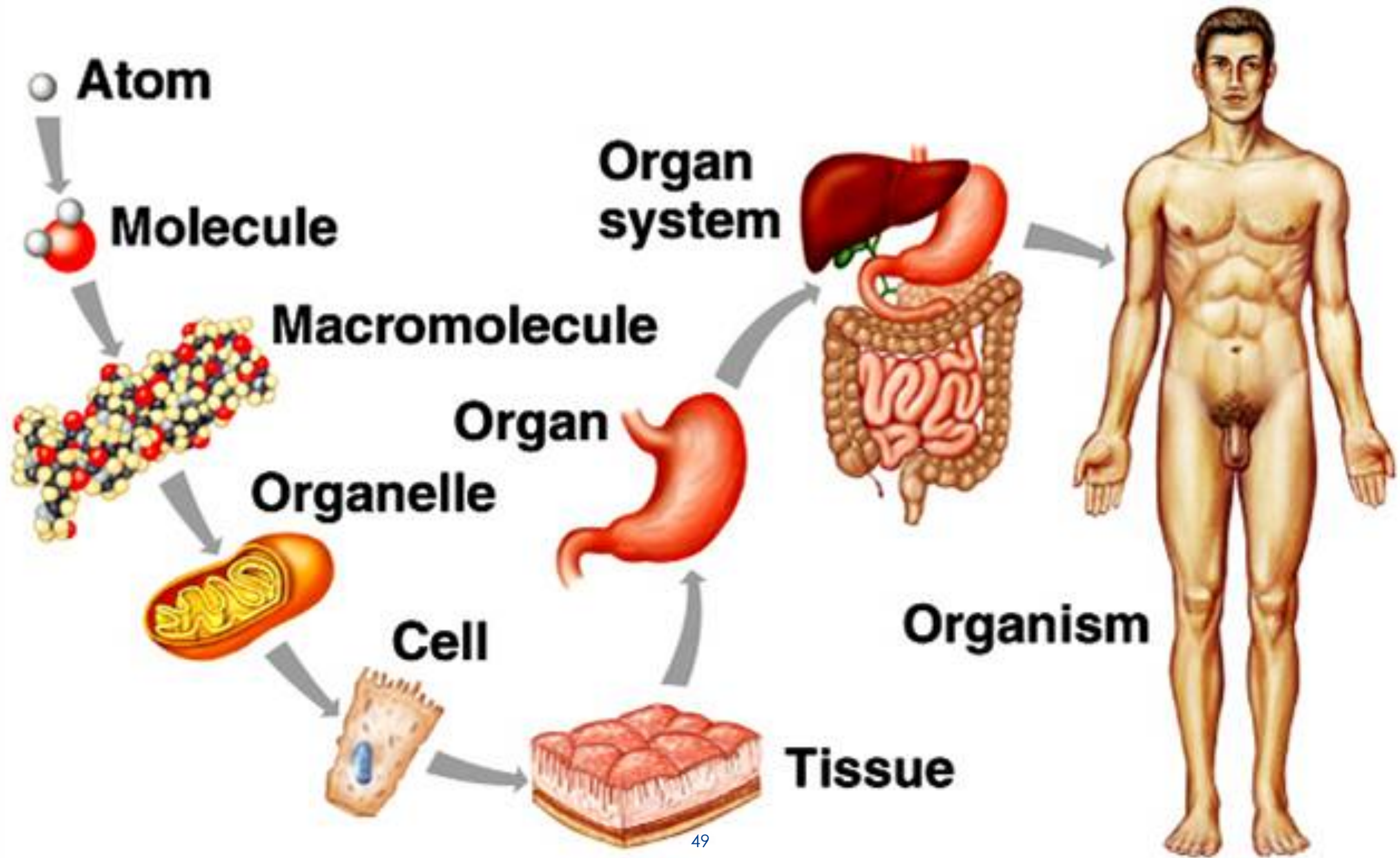
Characteristics of Blood

Functions of Blood

1. Transport – glucose, gases, wastes, hormones, amino acids, vitamins, minerals, lipids
2. Regulation – body temperature, pH
3. Protection – clotting, immunity
4. Communication – hormones and other chemical messengers

Physical characteristics of blood

- * pH: 7.35 – 7.45
- * 8% of total body weight
 - * 5-6 liters in men
 - * 4-5 liters in women
- * Osmolarity: 280-300 mOsm/L
- * Temperature: 100.4 degrees F
- * More viscous than water
- * Tissue: ?



Blood Lab Testing

Basic Concepts and Principles

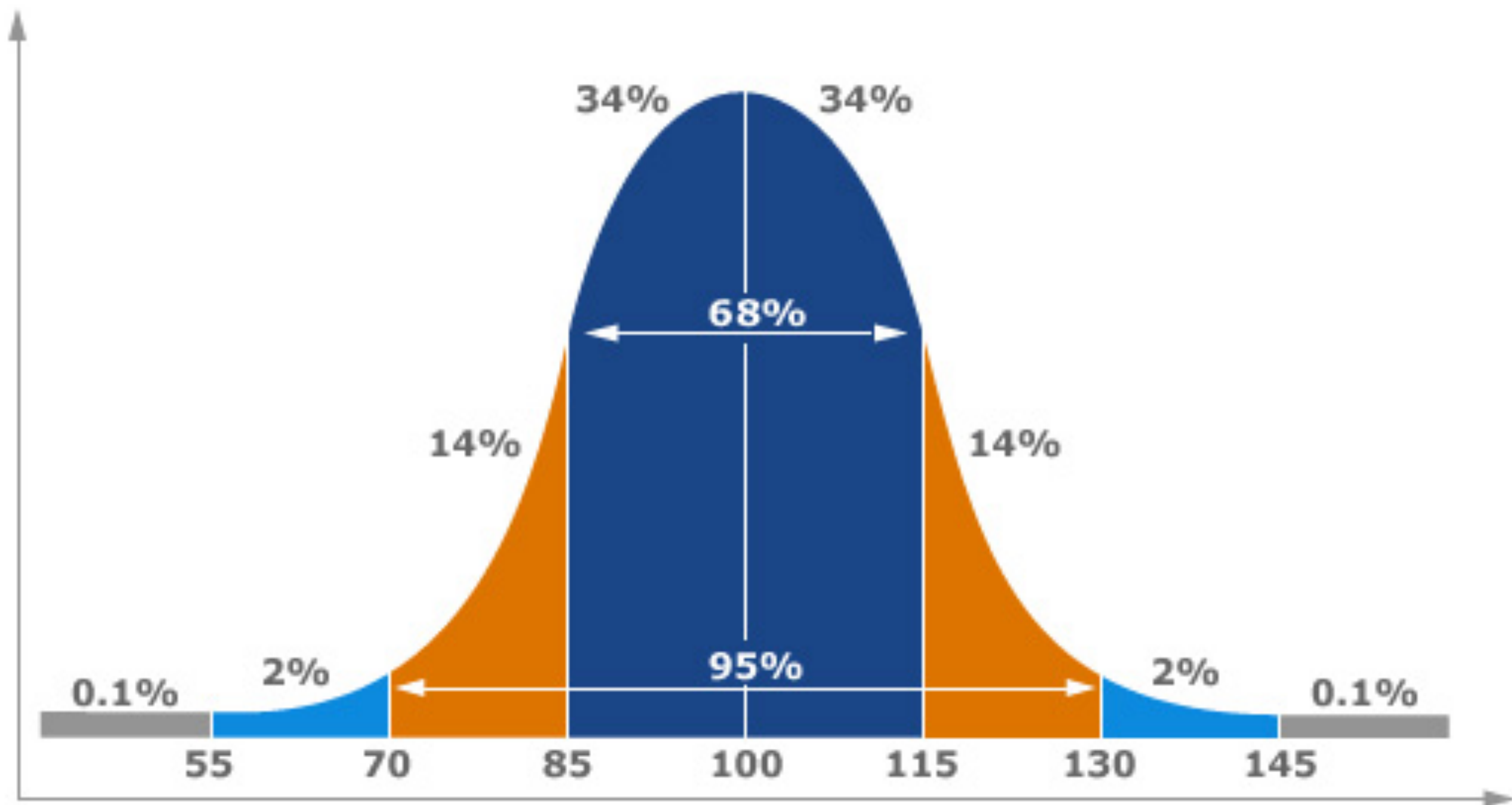
- * Purpose of lab testing is to reduce uncertainty and improve clinical decision making
 - * Offers objective data to use in conjunction with other gathered information
 - * Confirm suspicions taken during history and intake
 - * Guides or modifies therapy
 - * Assessment tool to evaluate effectiveness of client progress and therapies

Basic Concepts and Principles

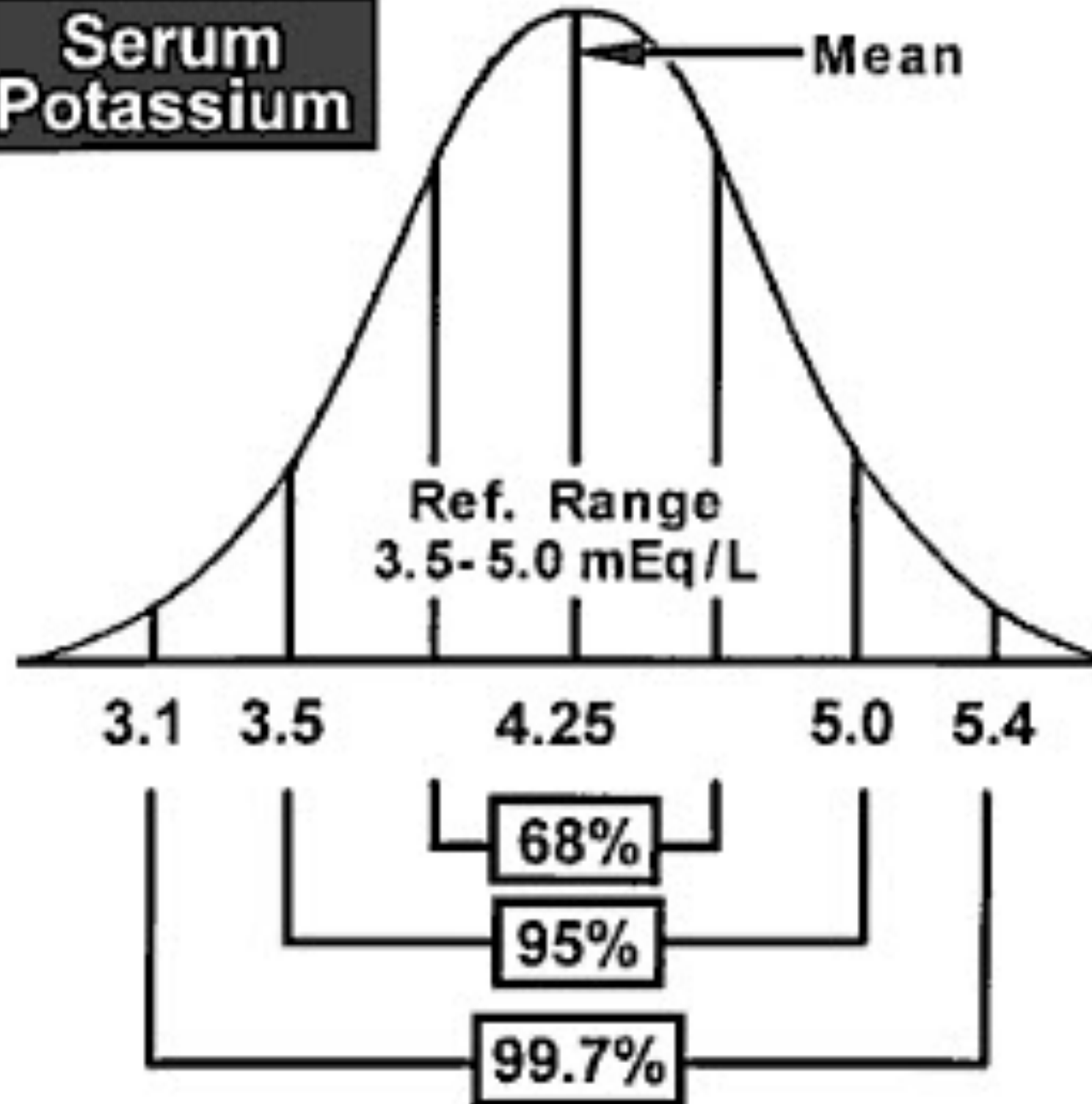
- * There is no such thing as a perfect test.
 - * Multiple factors to consider including:
 - * Sensitivity/specificity
 - * Hydration, tourniquet, diurnal rhythm
 - * Other confounding variables (eg glucose and vitamin C, medications)
 - * Multiple physiological processes happening simultaneously can make some markers appear normal

Reference Ranges

- * Most important aspect of laboratory test interpretation
 - * Dictates diagnosis and intervention
 - * Statistically derived numerical range based on sample population
-
1. Patients in specific geographic location
 2. Create a random (Gaussian) distribution curve
 3. Obtain the mean
 4. Calculate deviations from the mean



Serum Potassium



“The working group recognizes the reality that, in practice, very few laboratories perform their own reference interval studies.”

“Instead of performing a new reference interval study, laboratories and manufacturers refer to studies done many decades ago, when both the methods and population were very different.”



The recommendation is to select a statistically significant group (minimum of 120) of healthy reference subjects.

“Health is a relative condition lacking a universal definition. Defining what is considered healthy becomes the initial problem in any study....”



Recruiting a valid group of reference subjects and obtaining informed consent in today's environment is costly, time-intensive, and virtually an impossible task for most laboratories. Therefore, most laboratories do not establish their own RIs, but rather choose to verify RIs that have been reported by the manufacturer or as established by another laboratory. This is a relatively simple study and requires only 20 healthy subjects to recruit.



	Fasting Glucose (ng/dL)	Deviation from mean m	Squared deviation from mean m
Subject 1	99	2.75	7.56
Subject 2	94	-2.25	5.06
Subject 3	94	-2.25	5.06
Subject 4	105	8.75	76.56
Subject 5	101	4.75	22.56
Subject 6	83	-13.25	175.56
Subject 7	101	4.75	22.56
Subject 8	106	9.75	95.06
Subject 9	85	-11.25	126.56
Subject 10	90	-6.25	39.06
Subject 11	103	6.75	45.56
Subject 12	94	-2.25	5.06
	Mean = 96.25 (m) n=12	Mean = 0.00	Standard deviation = 7.55 (s.d.)

Lower Limit = $96.25 - 1.8 \times 1.04 \times 7.55 = \mathbf{82}$

Upper Limit = $96.25 + 1.8 \times 1.04 \times 7.55 = \mathbf{110}$

Reference Range = 82 – 110 ng/dL

Optimal Range

A tighter set of ranges designed to help:

1. Identify underlying physiological issues that can help explain symptoms
2. Evaluate health rather than disease
 - * Catch potentially more serious disease processes earlier

Sodium

Laboratory reference range:

135 – 145 mmol/L

Optimal reference range:

139 – 142 mmol/L

Potential issues with normal ranges

1. Individuals with subclinical or undiagnosed disease are included in the data to establish normal values.
2. References calculated from non-random (non-Gaussian) requires calculations and therefore may be inaccurate
3. Reference ranges may change
4. At $\pm 2SD$ from the average means 5% of the normal population will fall above (2.5%) and below (2.5%) the range.
 - * If a wider range ($\pm 3SD$) was used, would increase specificity and decrease sensitivity (eg people with the disease but small fluctuations in test values might be missed)
5. What/who is normal today?

Sensitivity and Specificity

Sensitivity

- Ability of a test to detect a positive test in every patient with a given disease
- A test with 100% sensitivity will be positive in every patient with a specific disease
- 90% sensitivity will not accurately identify 10% of individuals with a specific disease (false negative)
- The higher the sensitivity, the lower the chance of false negatives.
- PKU test is highly sensitive for infants with PKU. PSA test for prostate issues has a low sensitivity (~40%)

Sensitivity and Specificity

Specificity

- Percent of negative results in people without the disease
- Helps predict false positives
- Refers to how well a test successfully (specifically) identifies a given disease (only found in people with the disease)
- A test with 90% specificity may be positive in 10% of the people that *do not* have the disease
- The lower the specificity, the higher the chance for false positives

Sensitivity and Specificity - Example

“The Heart Attack Test” (THAT test)

Used on 200 people who have had a heart attack.

196 people tested positive (98% sensitivity)

- Four false negatives (people had it, but weren't identified)

Used on 200 people who did *not* have a heart attack

20 people tested positive (90% specificity)

- Identified the disease 10% of the time even though the disease wasn't present

Factors to Consider

- * Laboratory Error
 - * Equipment, human, calibration, sample prep, reagent discrepancies, drawing blood
 - * Tourniquet left on longer than one minute can increase protein, iron, AST, and cholesterol, and decrease potassium and creatinine
- * Laboratory reference range
- * Hydration status
- * Diurnal rhythms
 - * TSH, ALT, GGT, LDH, uric acid, phosphorus, calcium, hemoglobin, hematocrit, iron, certain WBCs
- * Altitude
- * Age, race, gender
- * Fasting (or starving?)
- * Medications
- * Nutritional supplements
- * Exercise
- * Diet

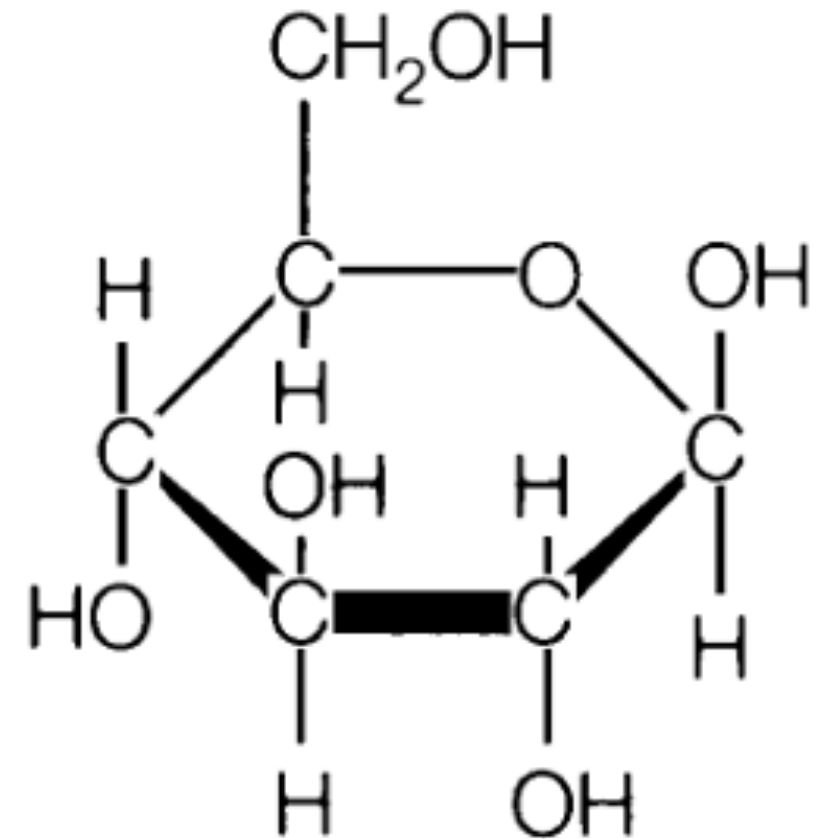
- 
- * The majority of blood chemistry markers, when out of range, merely point to a list of possibilities
 - * Combining this information with history, intake, signs/symptoms, and other data can help narrow to a list of probabilities
 - * Rarely will a lab test dictate the course of treatment
 - * Offers additional insights

Section Break Number 2

Glucose Regulation and Relevant Markers

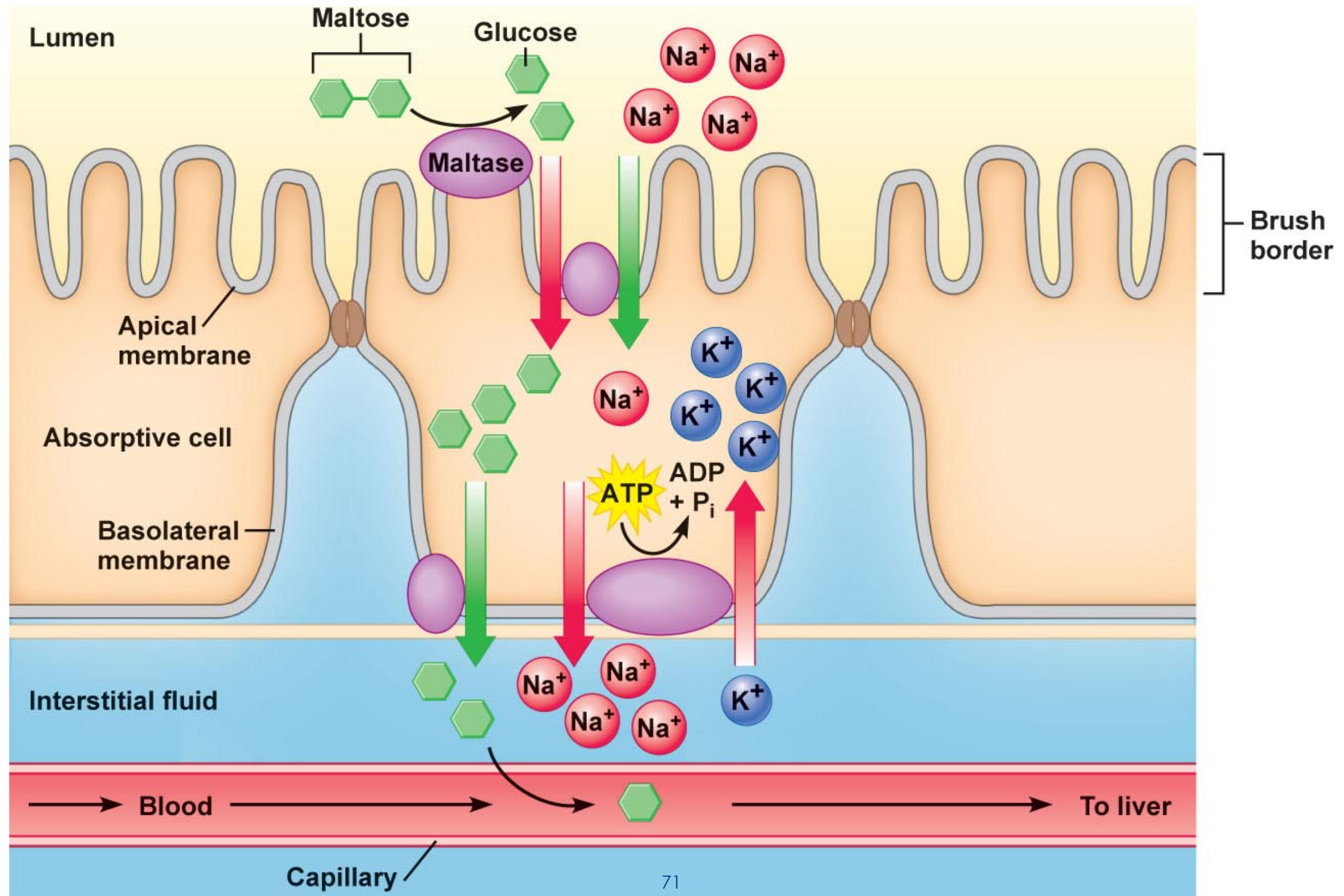
Glucose

- * Primary substrate for ATP production within cells
 - * Glycolysis, CAC, ETC/OP
 - * Other substrates can be used, eg fatty acids, amino acids
- * Also used for glycolipids & glycoproteins



Digestion and Absorption

- * Consumed as polysaccharides (Eg starch), oligosaccharides (eg stacchiose), disaccharides (eg sucrose, lactose), or monosaccharides (eg glucose, fructose)
- * Glycosidic bonds broken down by alpha-amylase (salivary, pancreatic)
- * Brush border enzymes (maltase, sucrase, lactase) break down dipeptides
- * Absorbed across brush border of enterocytes via Na dependent co-transport
- * Leaves enterocytes into hepatic portal circulation to the liver



Biochemically speaking

Insulin stimulates:

- Lipogenesis (fatty acid synthesis)
 - Triacylglycerol synthesis
- Cholesterol synthesis
- Glycogen synthesis
- Glycolysis

Glucagon stimulates:

- Lipolysis
- Gluconeogenesis
- Glycogenolysis

Serum glucose – where does it come from?

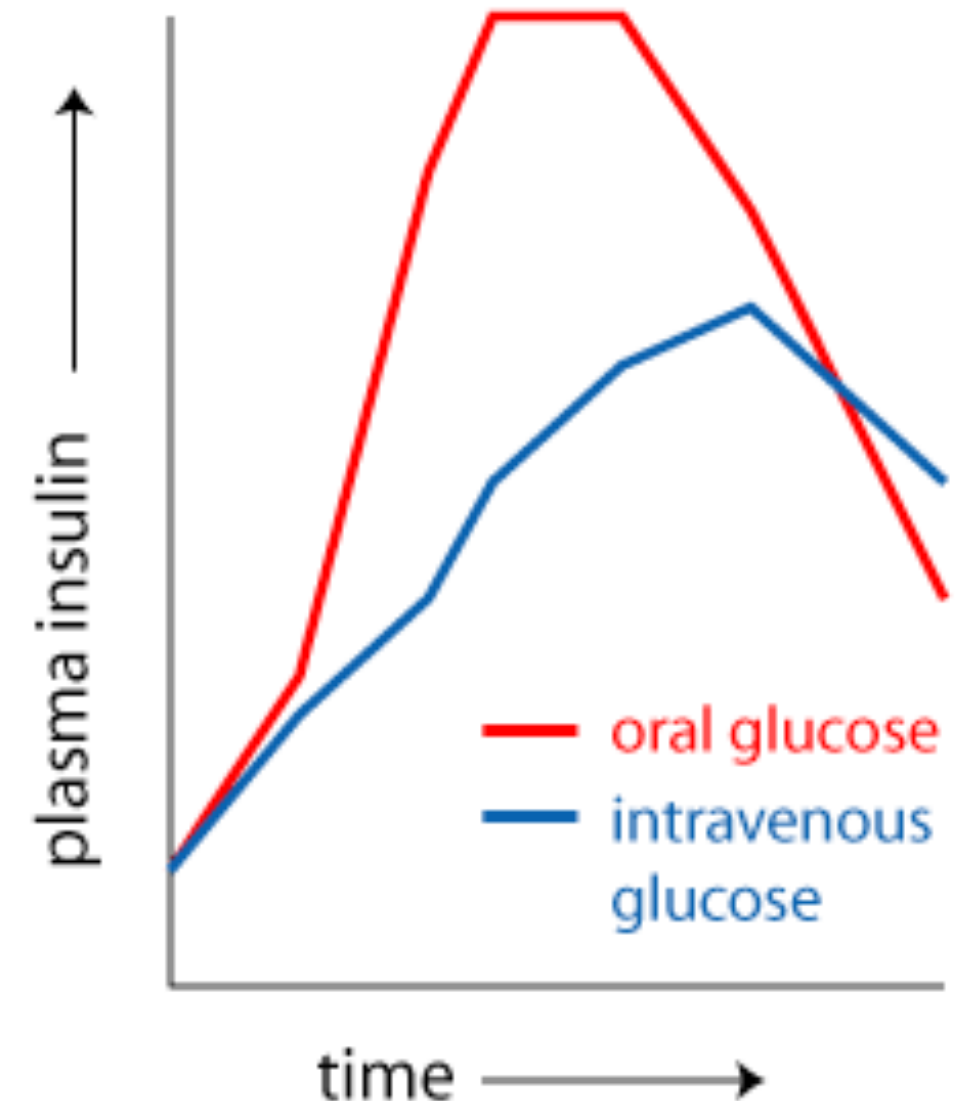
- * Food intake
- * Glycogenolysis (breakdown of glycogen)
- * Gluconeogenesis

Your goal is to maintain normal blood glucose levels



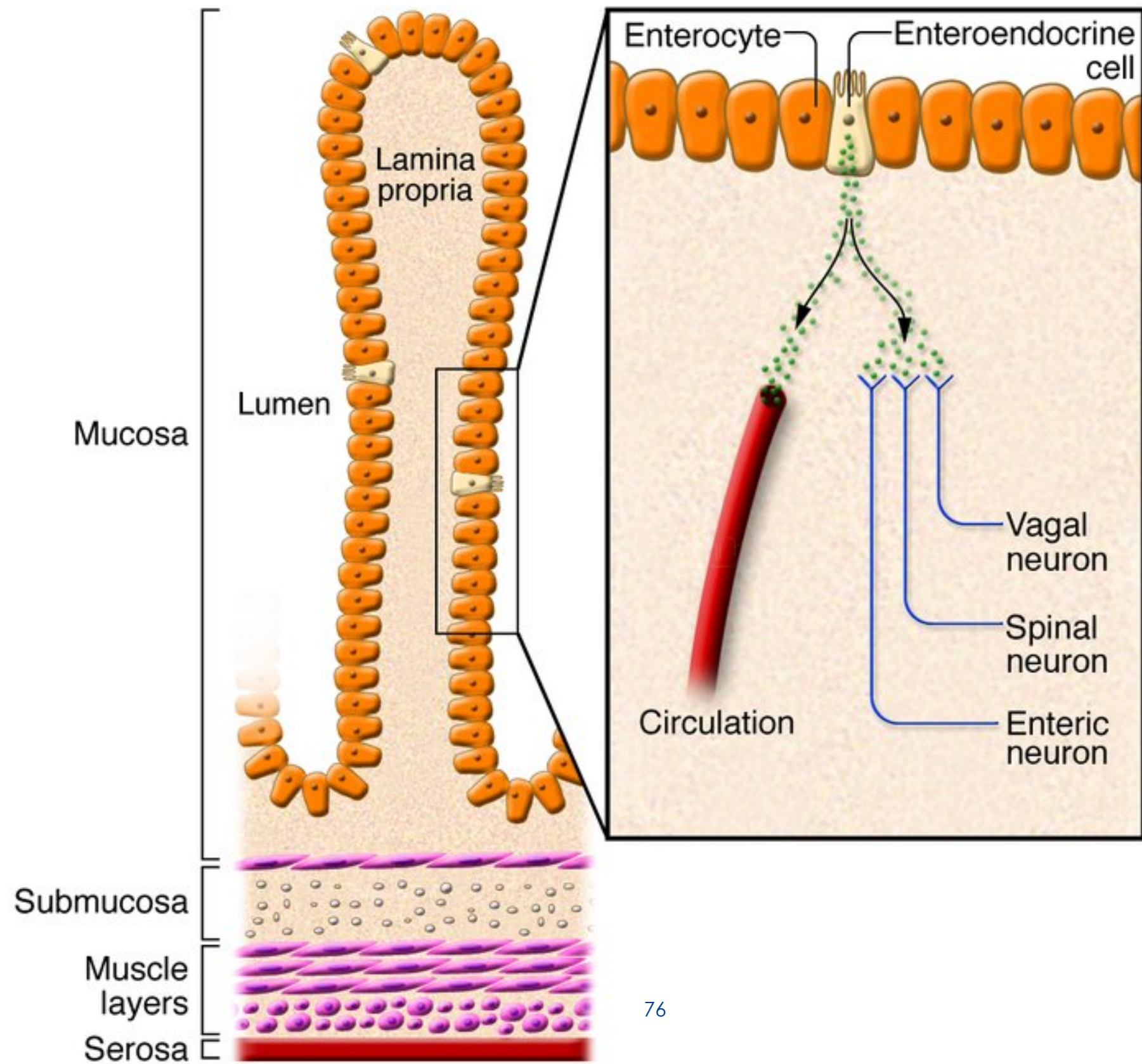
Incretins – Gut Hormones

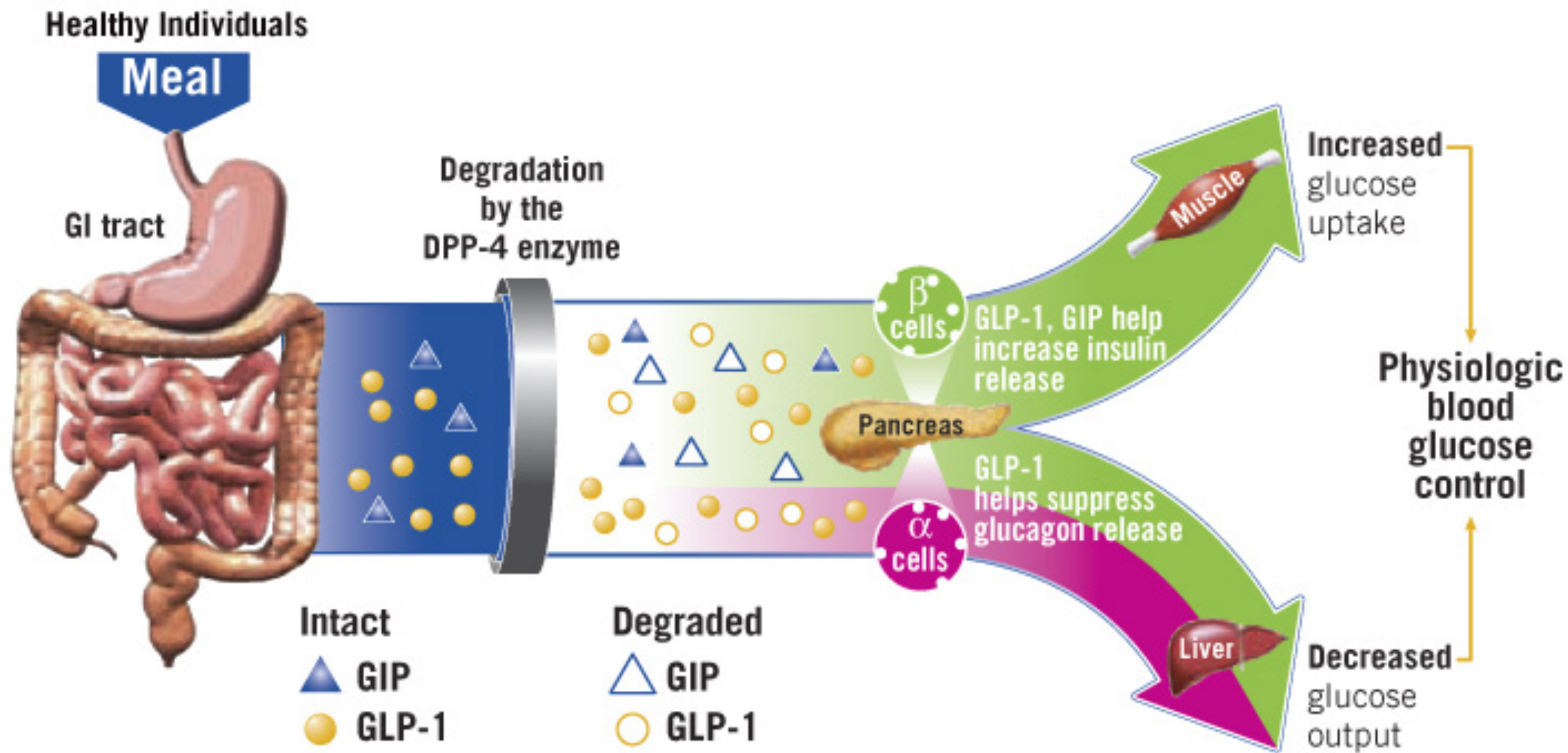
- * When glucose is present in the small intestine, regulatory hormones are released to stimulate insulin production *before* glucose enters the blood stream
- * Concept was developed when it was observed that oral glucose increased insulin levels more than intravenous glucose



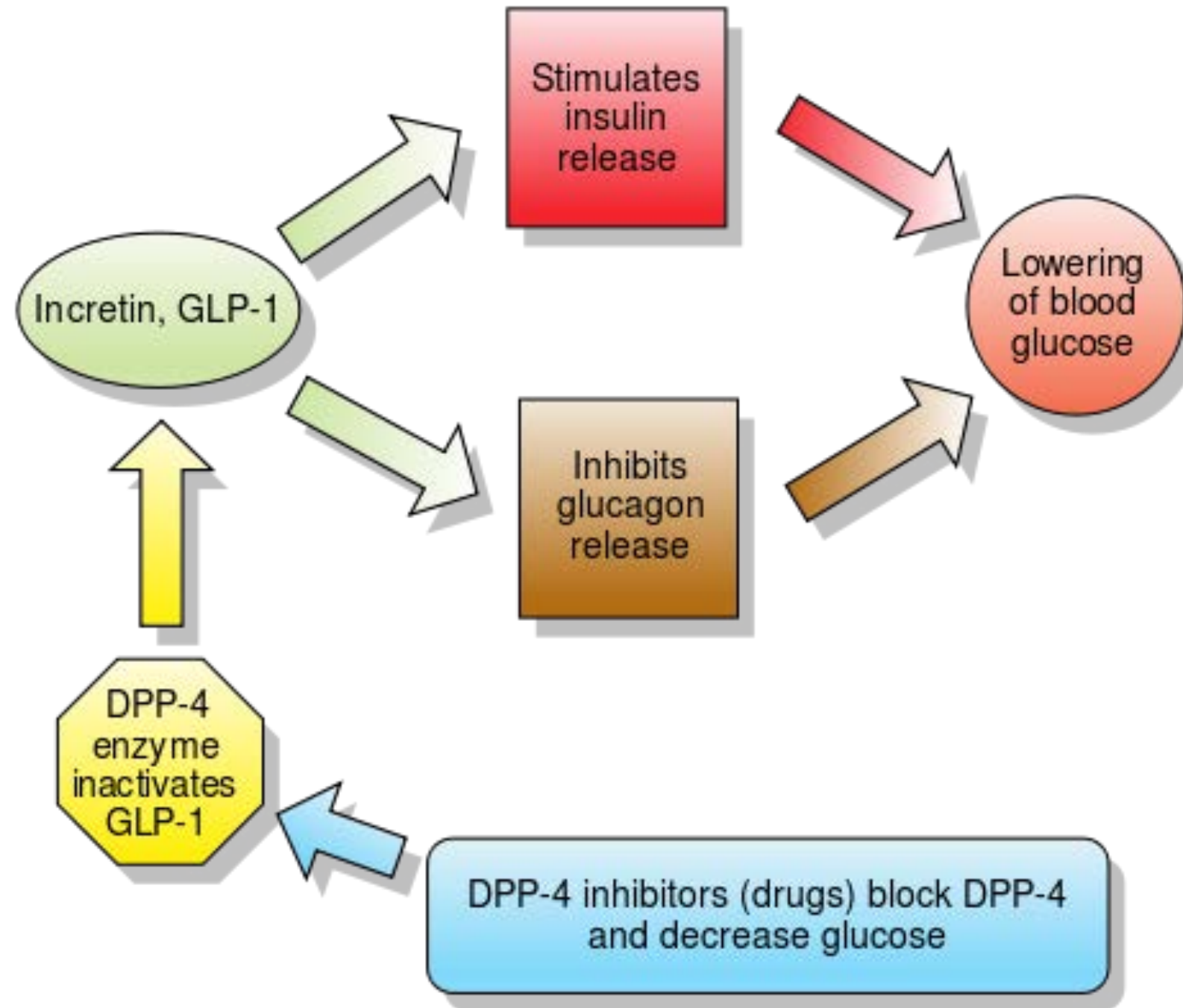
Incretins

- * Glucose-Dependent Insulinotropic Peptide (GIP)
 - * Previously gastric inhibitory peptide
- * Glucagon-Like Peptide-1 (GLP-1)
 - * Produced by enteroendocrine cells in the small intestine (duodenum)
 - * Both stimulate insulin secretion and decrease gastric motility
 - * Delays stomach emptying
 - * GLP-1 also inhibits glucagon, reduces food intake, and may increase number and mass of beta cells of the pancreas
- * Dipeptidyl Peptidase-4 (DPP-IV) breaks down GIP and GLP-1 (JANUVIA)





This illustration is an artistic rendition and is not necessarily representative of clinical effects.



Digestion and Absorption

- * When glucose enters circulation, pancreatic beta cells release and synthesize insulin
- * First phase insulin response – preformed insulin stored within the beta cells is released.
 - * Limits post-prandial glucose elevation
 - * Suppresses hepatic glucose production (gluconeogenesis)
 - * Begins within 2 minutes of nutrient ingestion, continues for 10-15 minutes
- * Second phase insulin response – insulin synthesis and release continues until serum glucose levels are normalized; longer process due to need to synthesize new insulin

First Phase Insulin Response

- * Diminished first phase insulin response considered to be one of the earlier predictive markers of metabolic syndrome and Type II Diabetes
 - * Occurs well before significant changes in absolute glucose concentrations
 - * May even occur prior to insulin resistance
- * Thought to indicate early beta cell dysfunction

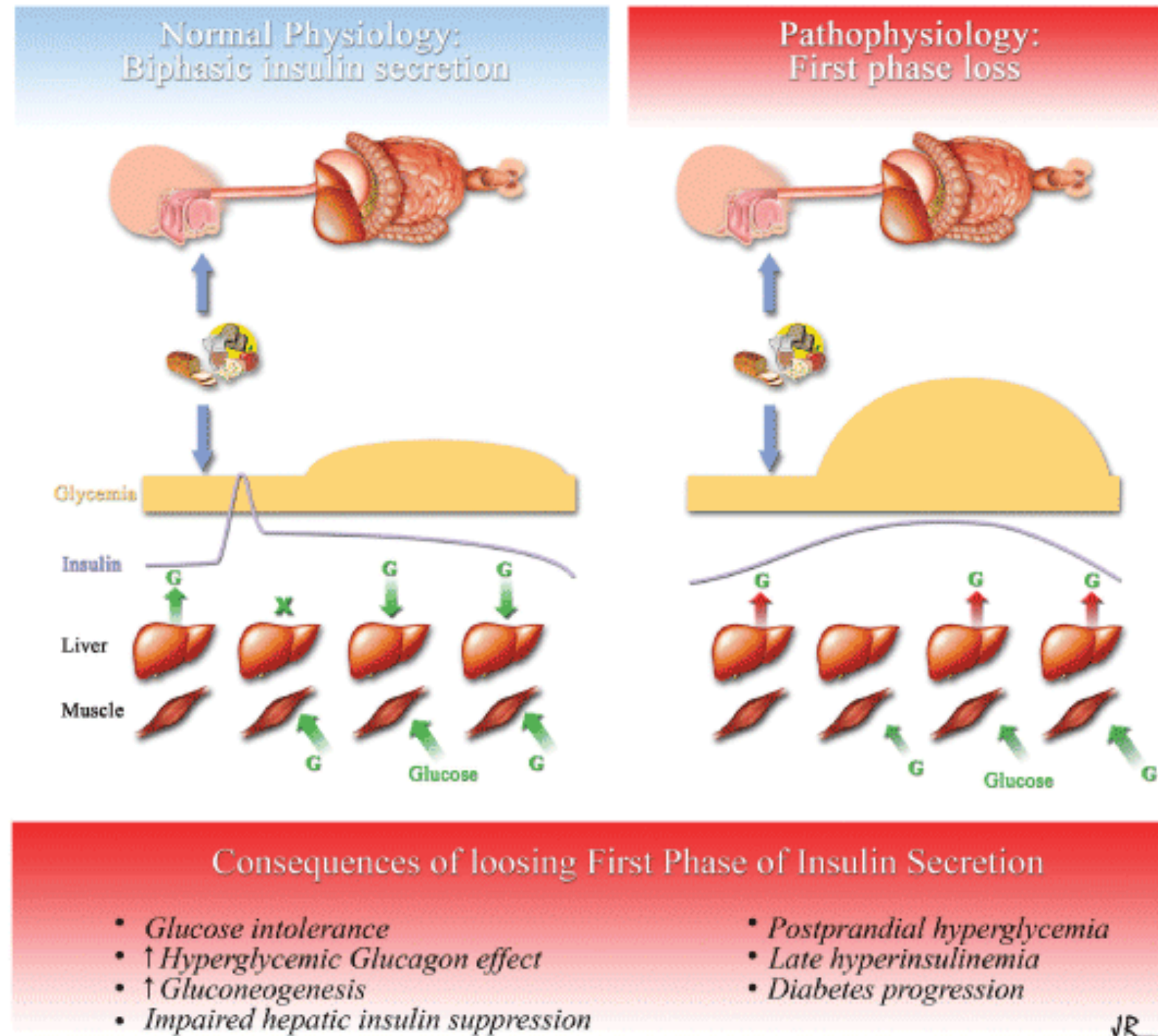


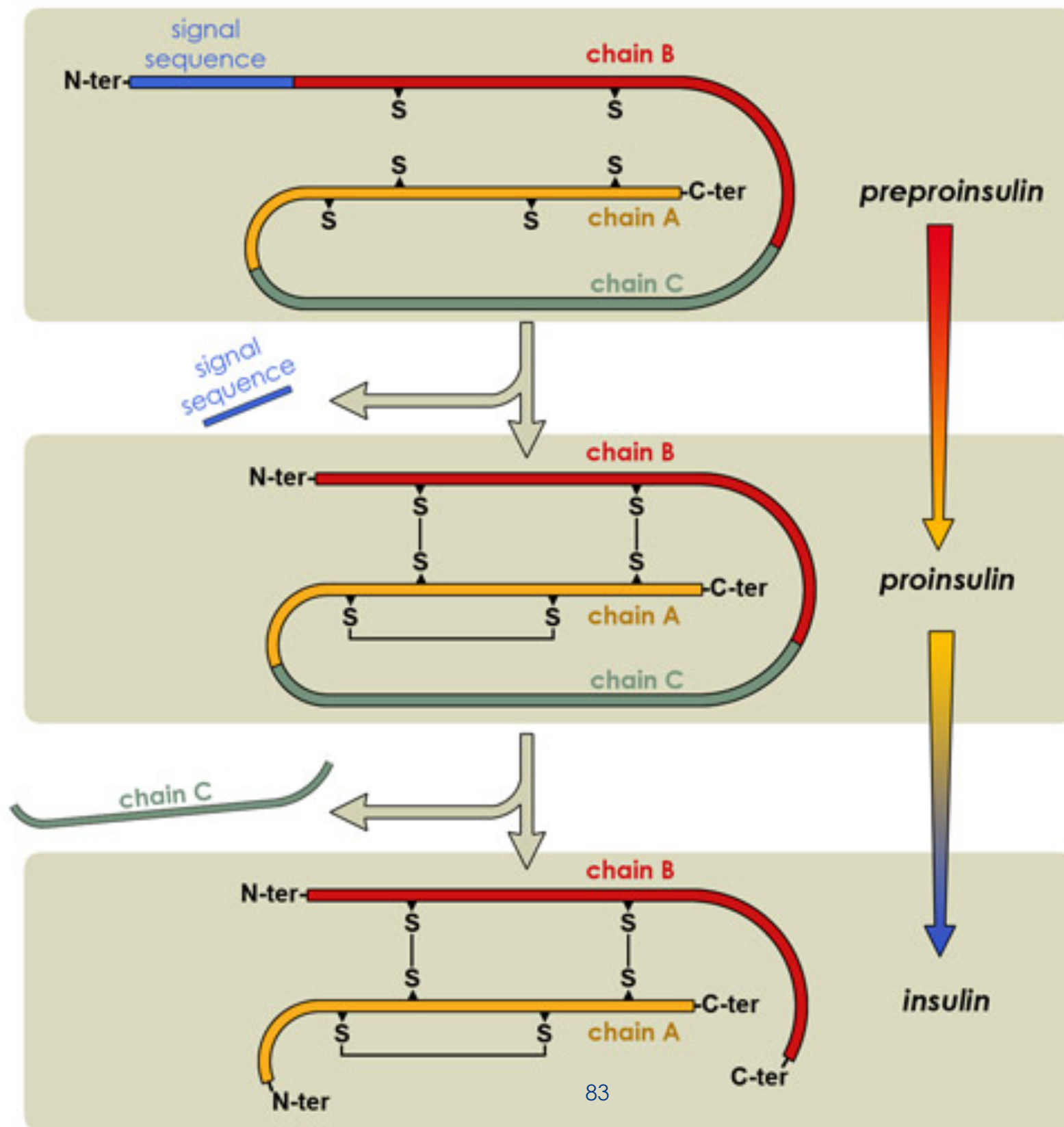
Fig. 1 - Effects of first phase failure: Sustained hepatic glucose output, reduced peripheral glucose uptake, resulting in postprandial hyperglycemic.

Insulin Synthesis

51 amino acid anabolic peptide hormone

Occurs within the beta cells of the pancreatic islets.

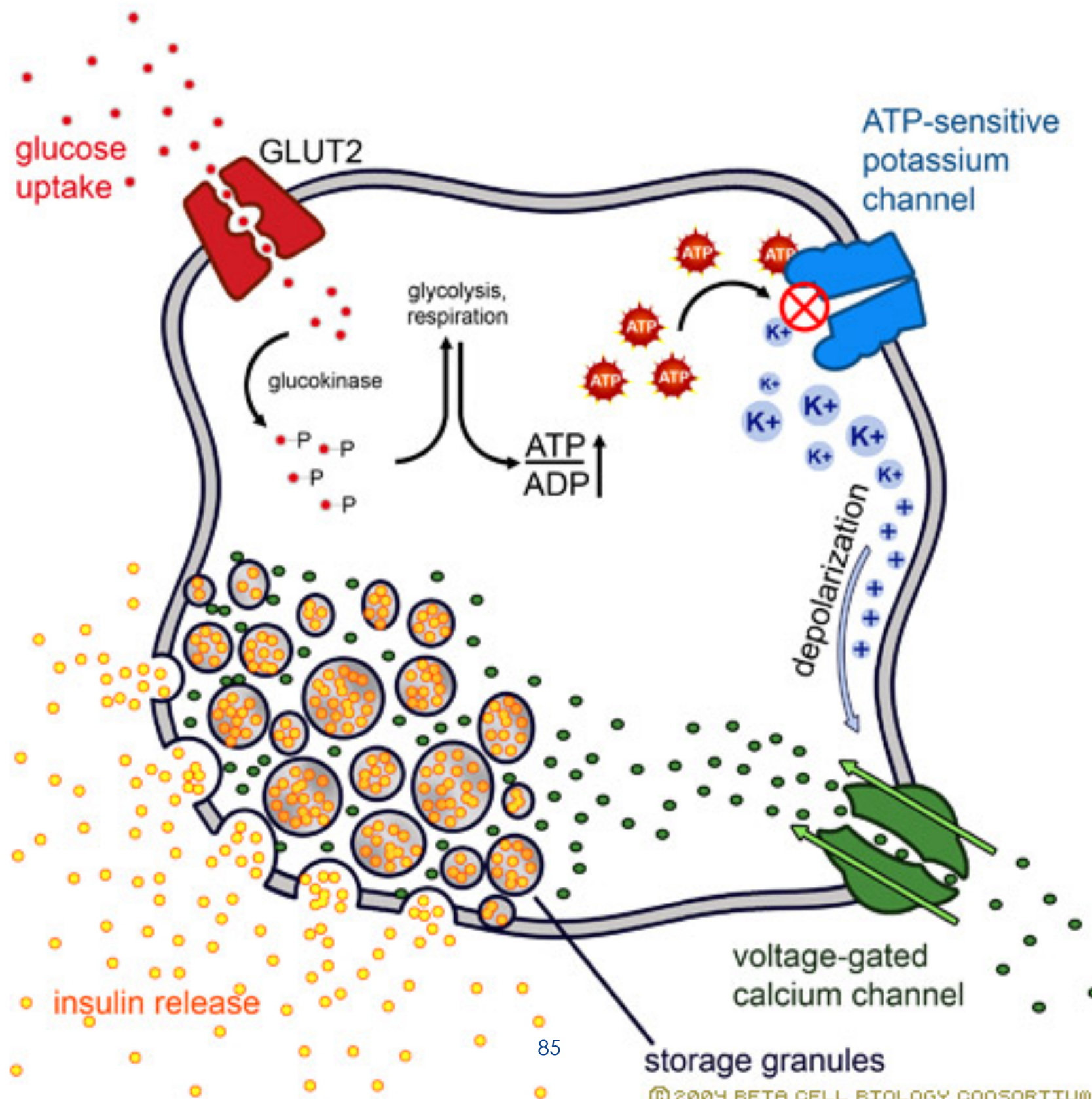
1. mRNA transcript creates a single chain precursor called preproinsulin
2. Preproinsulin enters the endoplasmic reticulum. Proteolytic enzymes cleave *signal peptide* to form proinsulin.
3. Proinsulin contains three domains
 1. Carboxy-terminal A chain
 2. Amino-terminal B chain
 3. Connecting peptide – C chain
4. Within the ER, endopeptidases cleave the C peptide, which is packaged and stored in the golgi apparatus and secretory vesicles until stimulated



Insulin Release

1. GLUT-2 transporters bring glucose into beta cells of the pancreas
2. This leads to an increase in ATP production (glycolysis, CAC, ETC)
3. Increased ATP stimulates the K⁺ATP Channel to block potassium ions, causing depolarization of the cell membrane*
4. The depolarizing cell membrane allows Ca²⁺ to enter via voltage-gated calcium channels, causing exocytosis of insulin-containing secretory vesicles

*Hypokalemia (low potassium) has been shown to decrease insulin production from the pancreas.



Insulin Actions

- * Suppresses pancreatic glucagon
- * Pancreatic insulin first goes to the liver via the hepatic portal system
- * In the liver, insulin stimulates glycogen storage, cellular respiratory pathways and depending on the level of glucose, fatty acid and triacylglycerol synthesis
- * NASH?

Glucagon

29 amino acid polypeptide hormone produced by alpha cells of pancreas

1. Low ATP within alpha cells stimulates Adenylate cyclase, increasing cAMP.
2. cAMP stimulates Protein Kinase A
3. Ca^{++} channels open, stimulating exocytosis of glucagon

Glucagon

Conversely, if ATP is elevated (due to glucose, free fatty acids, ketone bodies), the process is reversed, Calcium channels remain closed, and glucagon is not released.

Glucagon

Stimulators of glucagon

- Hypoglycemia
- Amino acids
 - Also stimulate insulin which, during a non-carbohydrate meal could result in hypoglycemia, thus glucagon is released to compensate
- Epinephrine (beta adrenergic receptors)

Inhibitors of glucagon

- Hyperglycemia
- Ketones, fatty acids (likely to help regulate ketogenesis)
 - If glucagon was elevated, it would increase glucose and inhibit ketogenesis

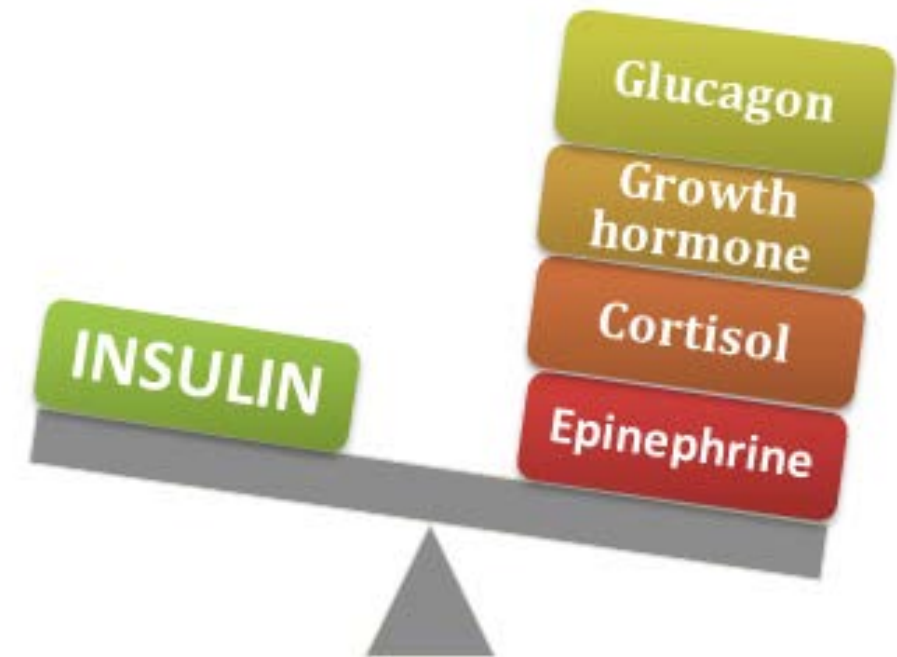
Glucagon Action

- * Increases:
 - * Glycogenolysis – increases glycogen phosphorylase, decreases glycogen synthase
 - * Gluconeogenesis – increases hepatic uptake of amino acids, which can be used by the liver to make glucose
 - * Lipolysis – liberating free fatty acids and thus ketogenesis (long term starvation)

Other glucose regulatory hormones

Anti Insulin / Counter regulatory hormones

- * Cortisol
- * Epinephrine/
Norepinephrine



Lab Testing – Glucose related markers

- * Fasting glucose
- * Hemoglobin A1C
- * C-Peptide (Insulin)
- * Glycomark

Fasting Glucose

- * Traditional Reference range:
 - * 65-100 mg/dL
- * Optimal Reference Range:
 - * 82 - 88 mg/dL

ORIGINAL INVESTIGATION

Open Access

Increased arterial stiffness in healthy subjects with high-normal glucose levels and in subjects with pre-diabetes

Jin Young Shin¹, Hye Ree Lee¹ and Duk Chul Lee^{2*}

Abstract

Background: Increased fasting plasma glucose (FPG), which includes impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and diabetes, is a risk factor for arterial stiffness. While IFG is widely accepted as a cardiovascular risk factor, recent studies have argued that subjects with high-normal glucose level were

FPG has an independent, positive association with ba-PWV in non-diabetic subjects after correcting for confounding variables, including age, sex, BMI, blood pressure, resting heart rate, hs-CRP, lipid profile, and behavioral habits.

An increase in FPG, even within the normal range, was associated with aggravated arterial stiffness.

FPG level for the prevention of cardiovascular risk is a topic of active research [5-7]. While IFG is widely accepted as a cardiovascular risk factor, recent studies have demonstrated that subjects with high-normal

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²Department of Family Medicine, Severance Hospital, Yonsei University College of Medicine, Seoul, Korea

Full list of author information is available at the end of the article



[11,12]. Brachial-ankle PWV (ba-PWV) is a suitable measure for screening for vascular dysfunction and the development of atherosclerosis in a preventive setting [13,14]. We assessed whether the FPG level is associated with arterial stiffness by measuring ba-PWV in non-diabetic healthy subjects with no history of cardiovascular disease, hypertension, or dyslipidemia.

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Q1 (n = 172), 65 - 83 mg/dL (low-normal)
Q2 (n = 188), 84 - 90 mg/dl (normoglycemia)
Q3 (n = 199), 91 - 99 mg/dL (high-normal)
Q4 (n = 138), 100 - 125 mg/dL (hyperglycemia)

Further research is needed to determine the glycemic target value for the prevention of arterial stiffness in clinical and public health settings.

Background

Increased fasting plasma glucose (FPG), which includes impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and diabetes, is a risk factor for arterial stiffness and cardiovascular disease [1-4]. Because there is no threshold glucose level to indicate the development of vascular disease, determining the appropriate target FPG level for the prevention of cardiovascular risk is a topic of active research [5-7]. While IFG is widely accepted as a cardiovascular risk factor, recent studies have demonstrated that subjects with high-normal

glucose level are characterized by a high incidence of cardiovascular disease [8-10]. This finding indicates that, even in the normal range, increased FPG can be associated with aggravation of arterial stiffness, which is considered an early marker of atherosclerosis.

Arterial stiffness can be easily and noninvasively assessed by measuring the pulse wave velocity (PWV) [11,12]. Brachial-ankle PWV (ba-PWV) is a suitable measure for screening for vascular dysfunction and the development of atherosclerosis in a preventive setting [13,14]. We assessed whether the FPG level is associated with arterial stiffness by measuring ba-PWV in non-diabetic healthy subjects with no history of cardiovascular disease, hypertension, or dyslipidemia.

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Fasting Blood Glucose: An Underestimated Risk Factor for Cardiovascular Death

Results from a 22-year follow-up of healthy nondiabetic men

JØRGEN V. BJØRNHOLT, MD

SIGURD NITTER-HAUGE, MD, PHD

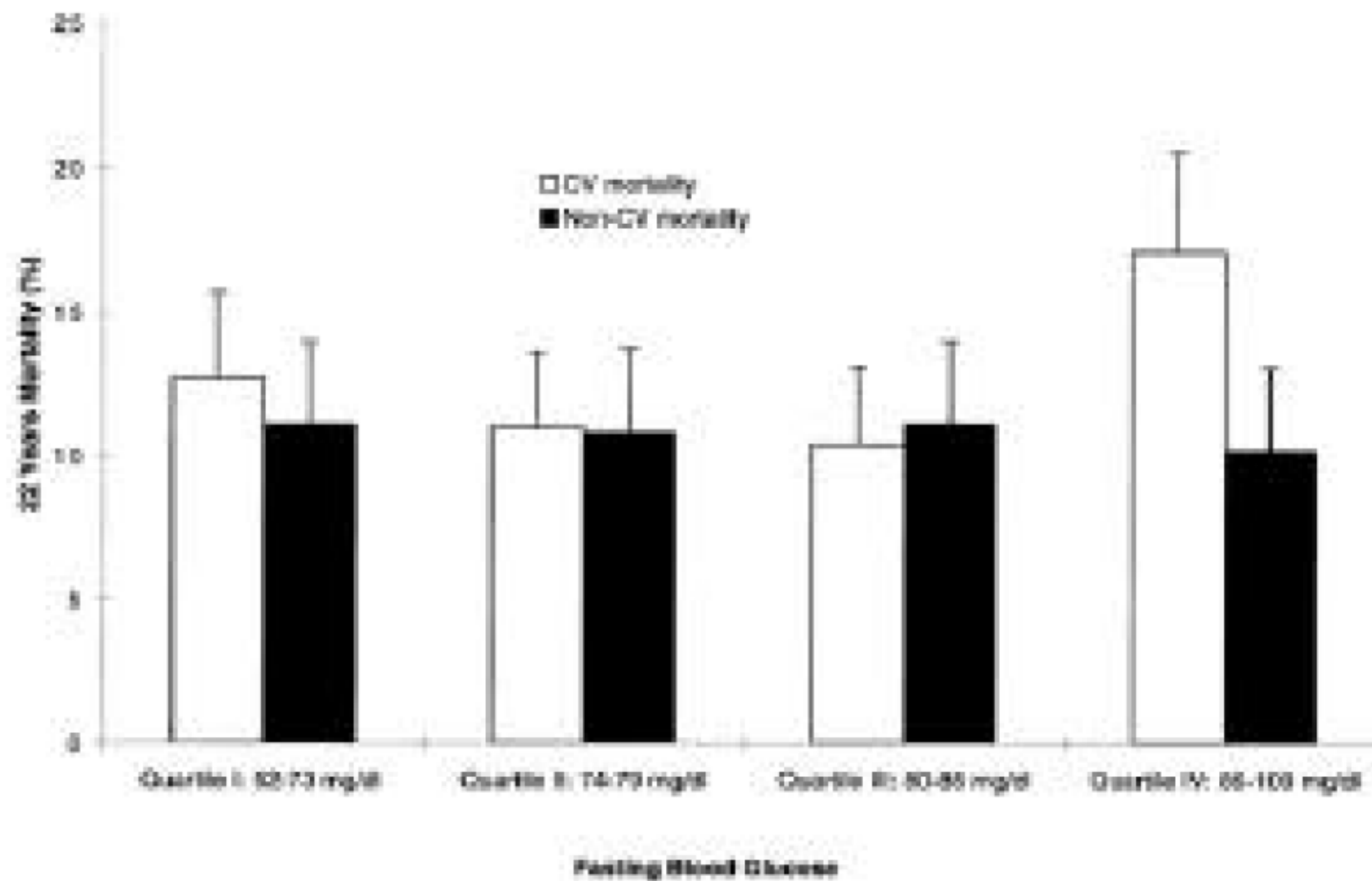
diabetes and either can be used diagnosti-

1,998 apparently healthy nondiabetic men (aged 40-59 years), a total of 1,973 with fasting blood glucose < 110 mg/dl were included in the study

After 22 years of follow-up, 483 men had died, 53% from cardiovascular diseases. After dividing men into quartiles of fasting blood glucose level, it was found that men in the highest glucose quartile (fasting blood glucose > 85 mg/dl) had a significantly higher mortality rate from cardiovascular diseases compared with those in the three lowest quartiles.

Q1: <73; Q2: 74-79; Q3: 80-85; Q4: 86 - 100

Fasting Blood Glucose: An Underestimated Risk Factor for Cardiovascular Death



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Received for publication 31 March 1998 and accepted in revised form 17 September 1998.

Abbreviations: ICD, *International Classification of Disease*; ROC, receiver operating characteristic; RR, relative risks.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

fasting and 8 h of abstaining from smoking. The examination program, described in detail elsewhere (11), included a comprehensive questionnaire on various health issues, a complete clinical examination, chest X ray, measurements of resting heart

Normal Fasting Plasma Glucose and Risk of Type 2 Diabetes Diagnosis

Gregory A. Nichols, PhD, Teresa A. Hillier, MD, MS, Jonathan B. Brown, PhD, MPP
Center for Health Research, Kaiser Permanente Northwest, Portland, Oregon.

ABSTRACT

PURPOSE: The study compares the risk of incident diabetes associated with fasting plasma glucose levels in the normal range, controlling for other risk factors.

METHODS: We identified 46,578 members of Kaiser Permanente Northwest who had fasting plasma glucose levels less than 100 mg/dL between January 1, 1997, and December 31, 2000, and who did not previously have diabetes or impaired fasting glucose. After assigning subjects to 1 of 4 categories (<85,

46,578 members of Kaiser Permanente Northwest who had fasting plasma glucose levels less than 100 mg/dL.

Assigned subjects to 1 of 4 categories (<85, 85-89, 90-94, or 95-99 mg/dL)

We followed them until they developed diabetes, died, or left the health plan, or until April 30, 2007.

Requests for reprints should be addressed to Gregory A. Nichols, PhD, Center for Health Research, 3800 N. Interstate Avenue, Portland, OR 97227-1098.

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that fasting plasma glucose levels significantly increased diabetes risk among young men (aged 26-45 years) with fasting plasma glucose levels less than 100 mg/dL.⁷ We recently reported that fasting plasma glucose independently increased diabetes risk among a community-based sample

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Compared with those with fasting plasma glucose levels less than 85 mg/dL, subjects with glucose levels of 95 to 99 mg/dL were 233% more likely to develop diabetes. Subjects in the 90 to 94 mg/dL group were 49% more likely to progress to diabetes.

Each milligram per deciliter of fasting plasma glucose increased diabetes risk by 6%.

paired fasting glucose to 100 mg/dL to better predict diabetes development.⁴

The reduction of the impaired fasting glucose cut-point generated international controversy, in part because the re-

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More recently, an Israeli study that adjusted for a number of other risk factors (family history of diabetes, smoking, hypertension, physical activity levels, triglycerides, and the ratio of total to high-density lipoprotein cholesterol) found that fasting plasma glucose levels significantly increased diabetes risk among young men (aged 26-45 years) with fasting plasma glucose levels less than 100 mg/dL.⁷ We recently reported that fasting plasma glucose independently increased diabetes risk among a community-based sample

Impaired Fasting Glucose: How Low Should It Go?

JONATHAN E. SHAW, MRCP
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MAXIMILIAN DE COURTEN, MD

GARY K. DOWSE, MBBS
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K. GEORGE M.M. ALBERTI, DPHIL

OBJECTIVE — Impaired fasting glucose (IFG) has been recently introduced as a stage of abnormal carbohydrate metabolism, but the evidence on which its glucose limits (fasting plasma glucose [FPG] 6.1–6.9 mmol/l) are based is not strong. The aim of this study was to determine if 6.1 mmol/l represents a clear cutoff in terms of the risk of future diabetes and in terms of elevated cardiovascular risk factor levels, and to examine the use of other lower limits of IFG.

RESEARCH DESIGN AND METHODS — A population-based survey of the island of Mauritius was conducted in 1987, with a follow-up survey in 1992. On both occasions,

analogous to (although clearly different from) impaired glucose tolerance (IGT). This category has been supported by the World Health Organization (WHO) in a recent consultation document (4). Both the ADA and WHO have adopted the same criteria for IFG, an FPG ≥ 6.1 mmol/l and < 7.0 mmol/l, although studies now show that the individuals identified by these limits are mostly different from those identified as having IGT (5,6).

In the few studies that have investigated nondiabetic FPG levels, only broad categories of FPG have been assessed, so that it is not possible from the published data to

The risk of developing hypertension at follow-up was greater for those people with baseline FPG $\geq$ or $= 6.1$ mmol/l (110 mg/dL). The risk of developing diabetes at follow-up increased with increasing baseline FPG, but **there was little evidence of a threshold near 6.1 mmol/l.**

normal, in that they are associated with an increased risk of both macrovascular disease and future diabetes. The Paris Prospective Study reported that the risk of developing diabetes over 3 years was greater among middle-aged men with a fasting plasma glu-

among people with FPG 5.8–6.9 mmol/l (2). Evidence from such studies has led to the introduction of impaired fasting glucose (IFG) by the American Diabetes Association (ADA) (3), as a stage in the natural history of disordered glucose metabolism,

(OGTT), and investigated cardiovascular risk factors. Diabetes, almost universally type 2, was found in 11.9% of adults aged 25–74 years (7). A follow-up survey was performed in 1992, measuring the same parameters. This allowed us to assess the associations between a range of FPG values and parameters related to diabetes, both cross-sectionally and prospectively.

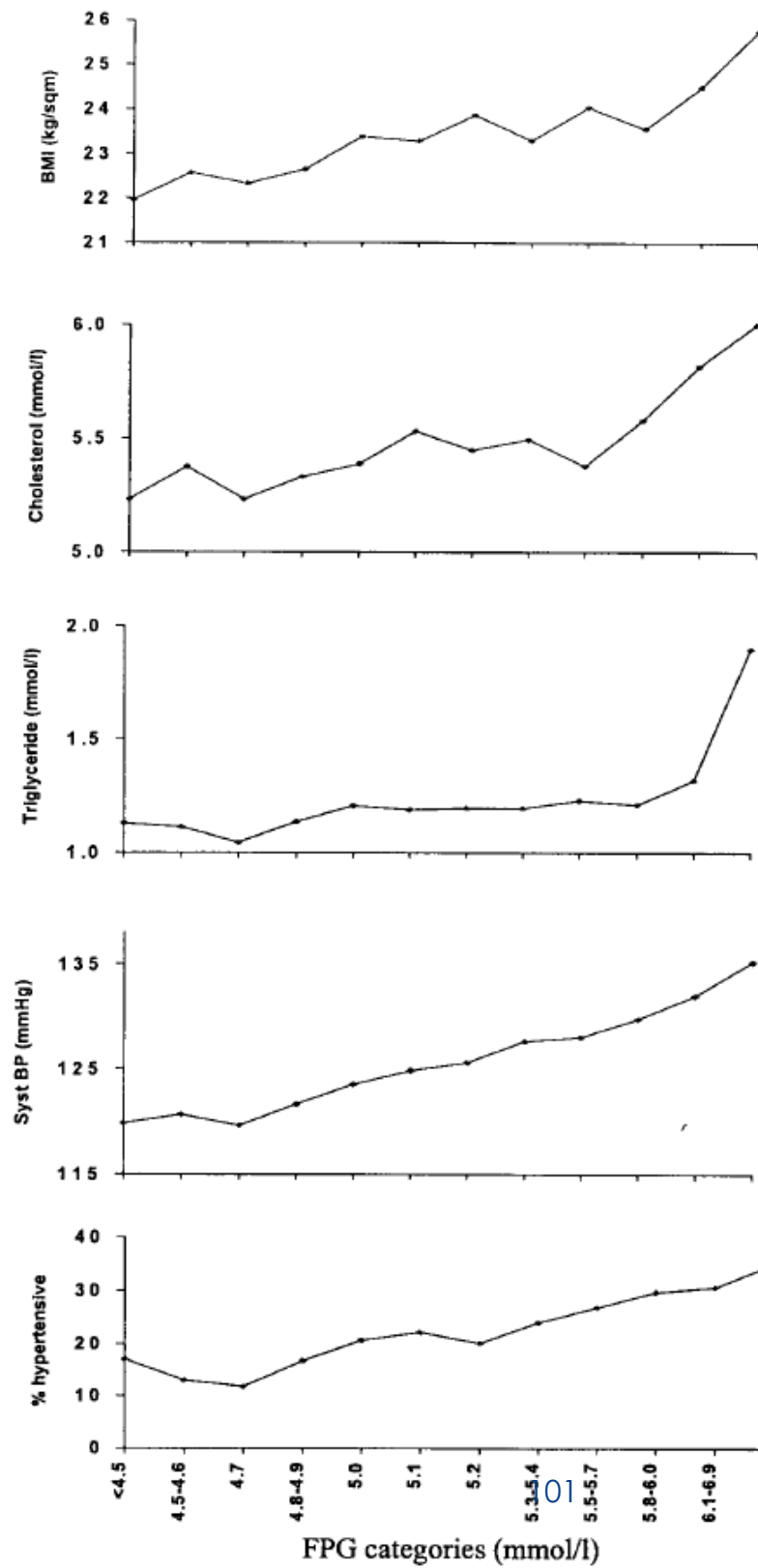
From the International Diabetes Institute (J.E.S., P.Z.Z., A.M.H., M.D.C., G.K.D.), Melbourne, Australia; the Ministry of Health (P.C.), Port Louis, Mauritius; the Department of Epidemiology and Health Promotion (J.T.), National Public Health Institute, Helsinki, Finland; and the Human Diabetes and Metabolism Research Center (K.G.M.M.A.), University of Newcastle upon Tyne, Newcastle upon Tyne, U.K.

Address correspondence and reprint requests to Dr. J. Shaw, Department of General Medicine, Wythenshawe Hospital, Southmoor Road, Wythenshawe, Manchester M23 9LT, U.K. E-mail: jshotham@hotmail.com. Received for publication 4 May 1999 and accepted in revised form 23 September 1999.

Abbreviations: 2-h PG, 2-h plasma glucose; ADA, American Diabetes Association; FPG, fasting plasma glucose; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; PPV, positive predictive value; ROC, receiver operator characteristic; WHO, World Health Organization; WHR, waist-to-hip ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

RESEARCH DESIGN AND METHODS — Mauritius is an Indian Ocean island nation ~ 800 km east of Madagascar. The population consists of $\sim 70\%$ Asian Indians, 2% Chinese, and 28% "general population" who are predominantly people of African ancestry (Creoles) with varying amounts of European, Malagasy, and Indian admixture. A population-based sur-



r*	p
0.15	<0.001
0.10	<0.001
0.17	<0.001
0.16	<0.001

Glucose

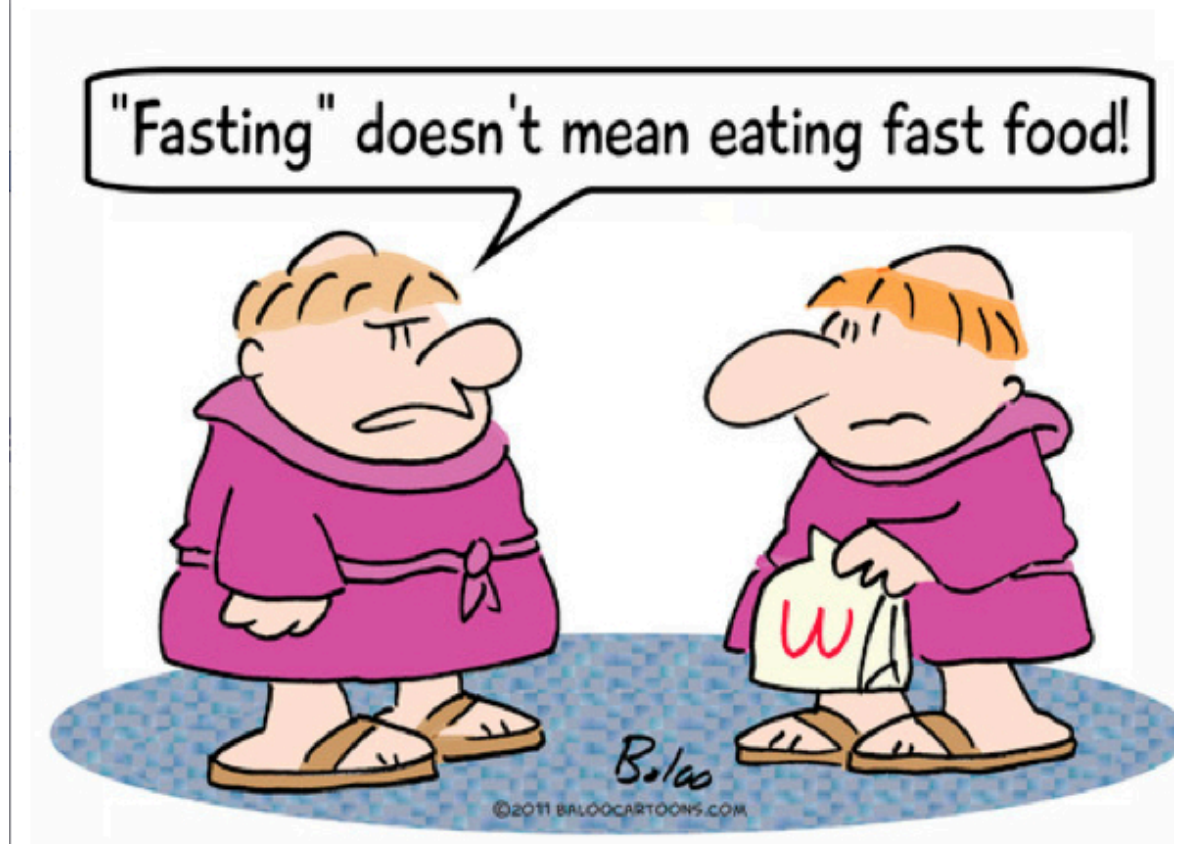
- * Traditional Reference range:
 - * 65-100 mg/dL
- * Optimal Reference Range:
 - * 82 - 88 mg/dL

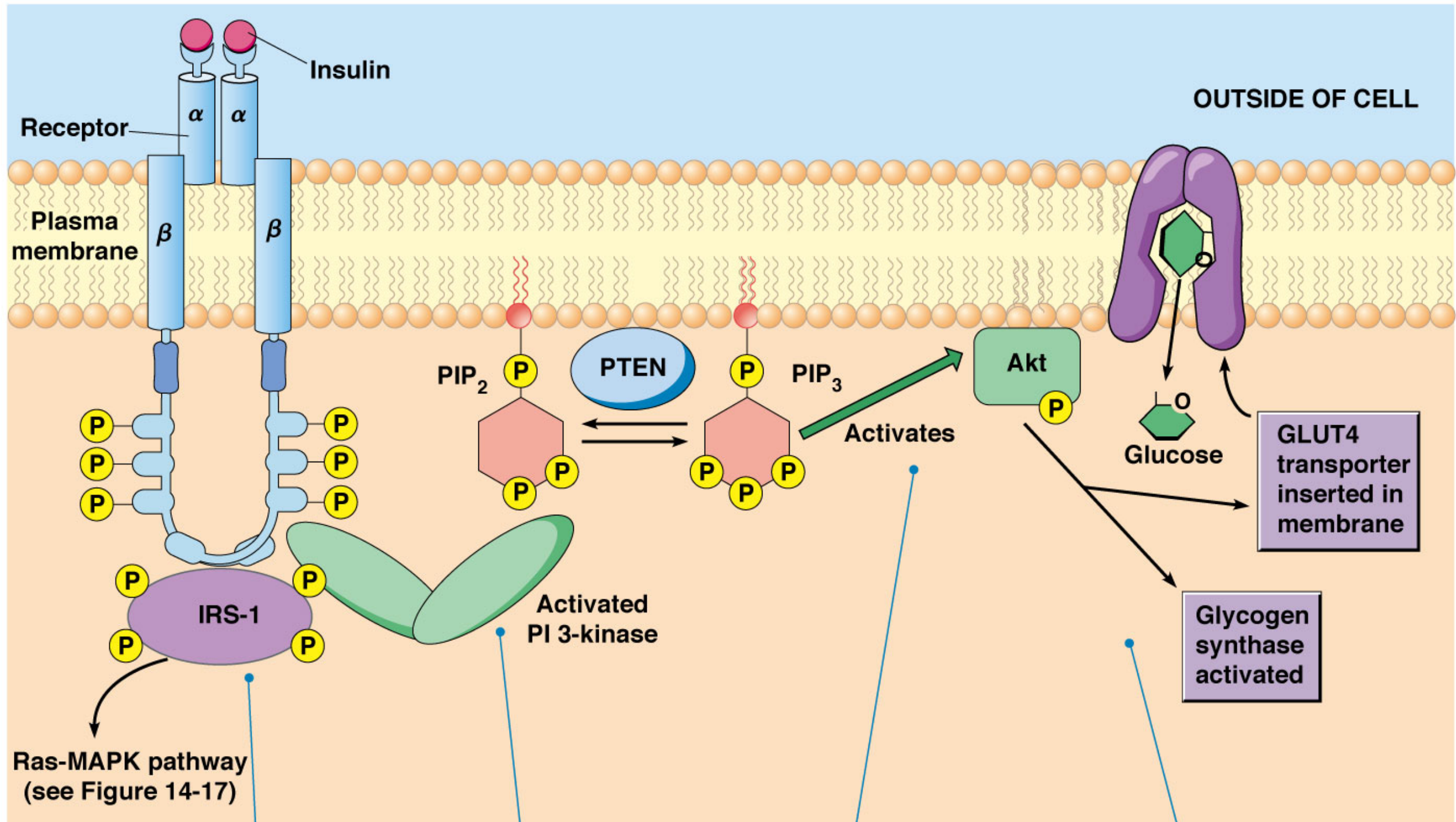
Glucose – Elevated

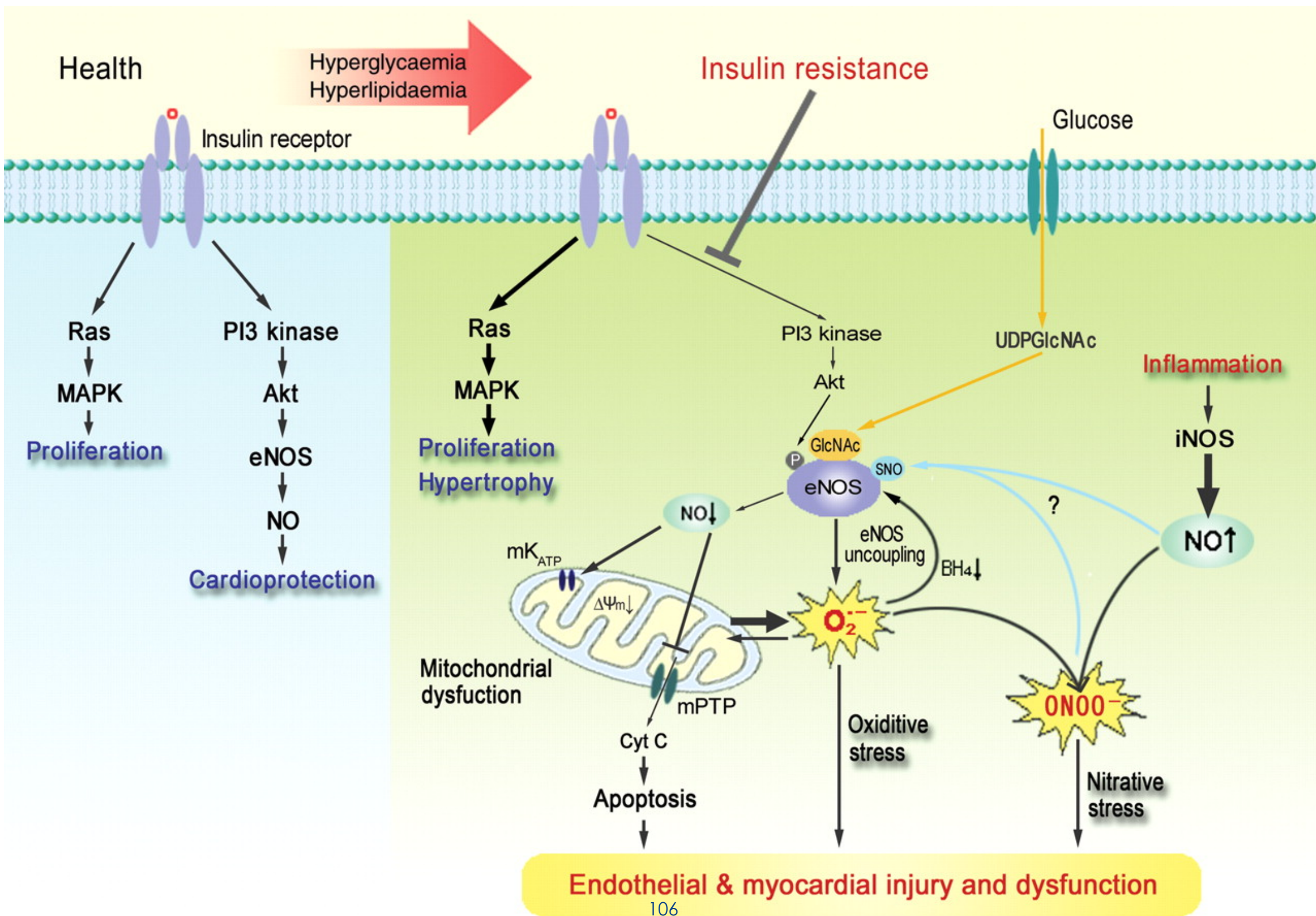
Cause	Reason	Additional Inquiry
Insulin resistance (increased glucagon)	Dysfunctional insulin signaling (eg receptors) causing glucose to remain elevated in the blood.	Fatigue after meals, sweets cravings even after carbohydrate ingestion; evaluate c-peptide, hemoglobin A1C
Pancreatic dysfunction	Poor insulin production	Evaluate c-peptide.
Hyperthyroidism	Shorter half-life of insulin, increased intestinal glucose absorption, increased FFA and thus hepatic gluconeogenesis, excess lactate leading to increased hepatic gluconeogenesis (Cori cycle), increase in GH, glucagon and catecholamines all promote hyperglycemia	Evaluate thyroid markers.
Hyperadrenal function	Increased cortisol and/or catecholamine increase glucose levels	Consider evaluating cortisol levels.

Glucose - Elevated

- * Must inquire about fasted state
 - * What time?
 - * Really fasting?
 - * 12-14 hours fasting?



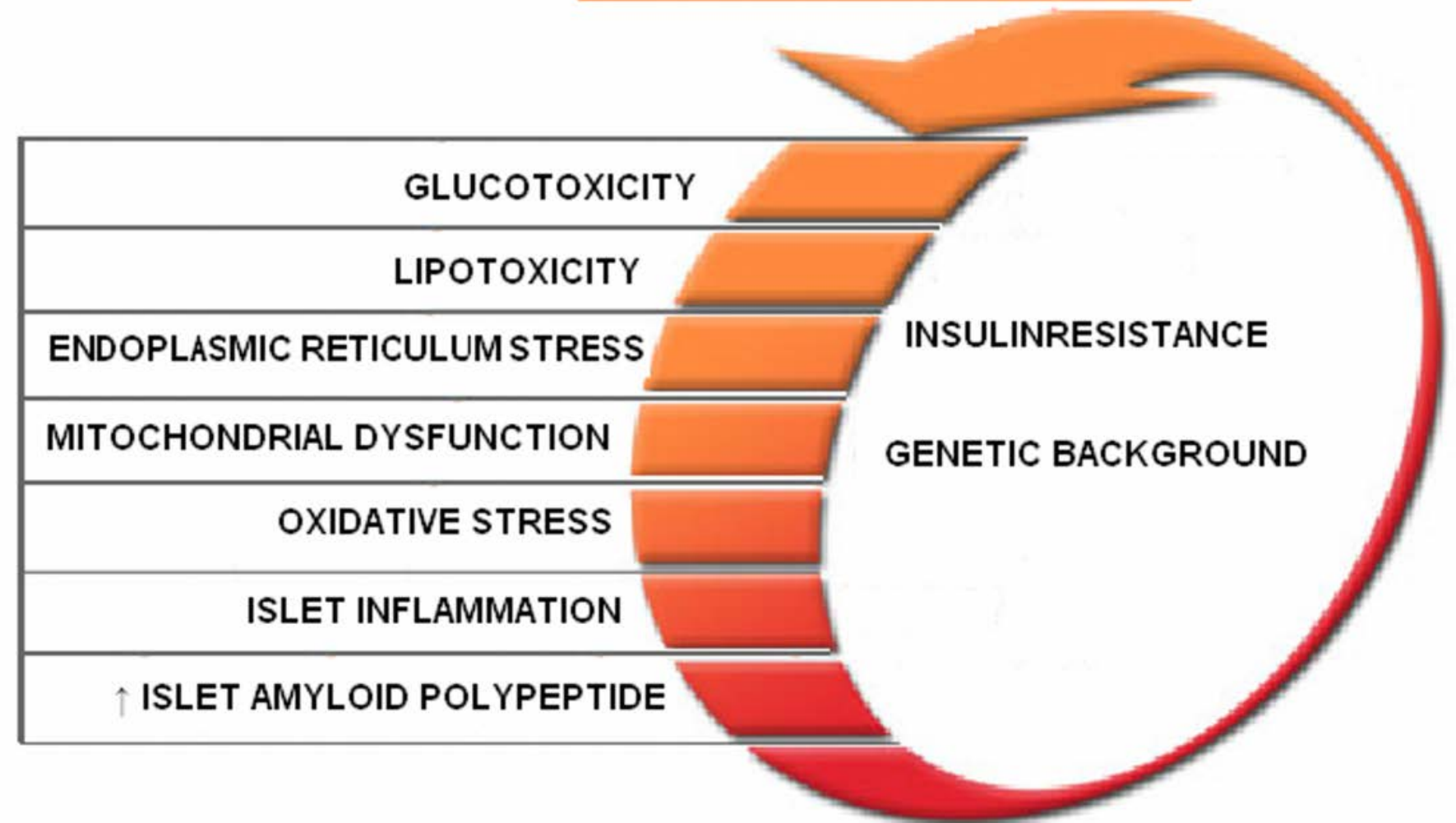




Pancreatic Dysfunction

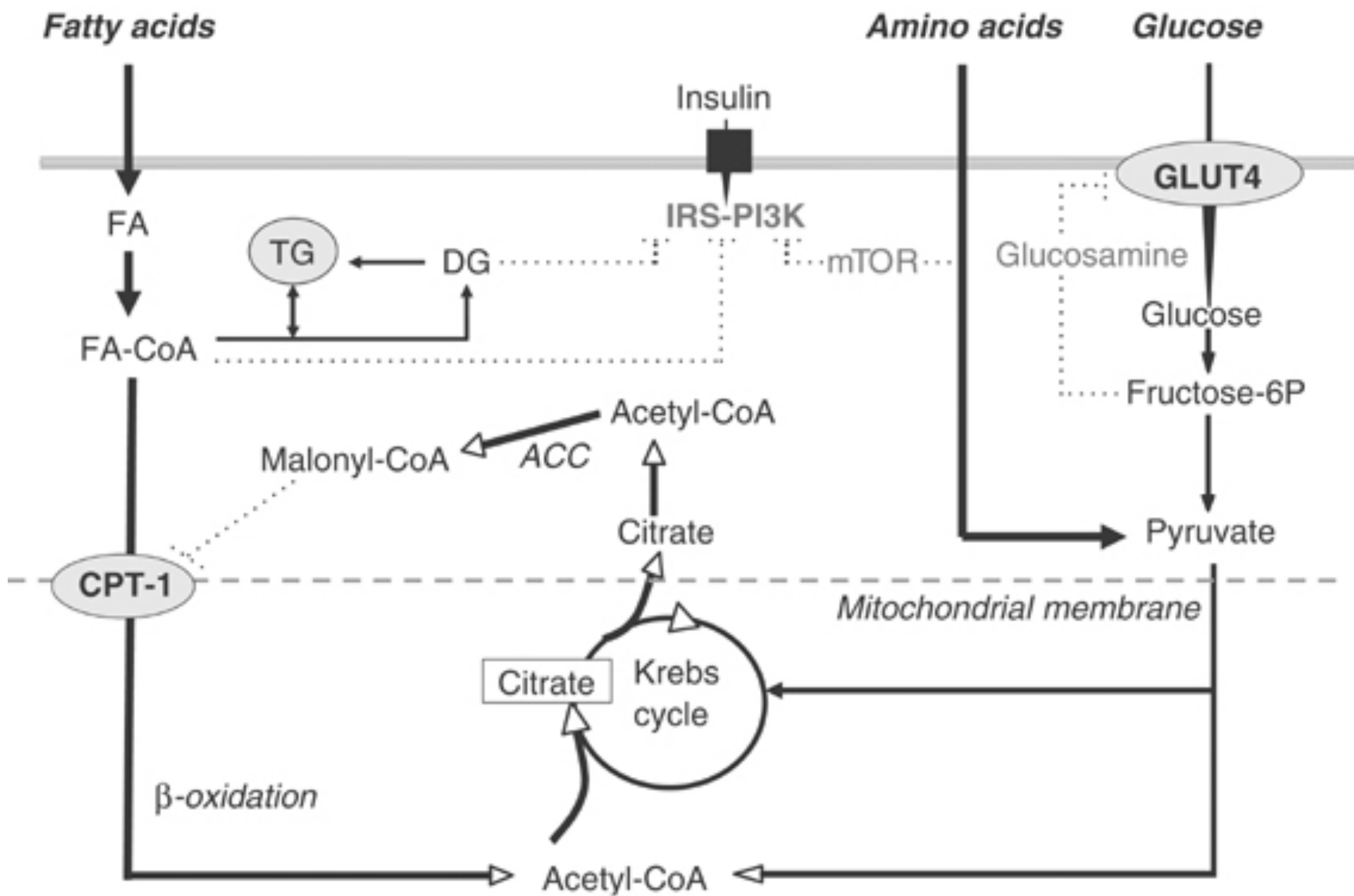
β-CELL FAILURE

- * Lipotoxicity
- * Glucotoxicity



Glucotoxicity and Lipotoxicity in Beta Cells

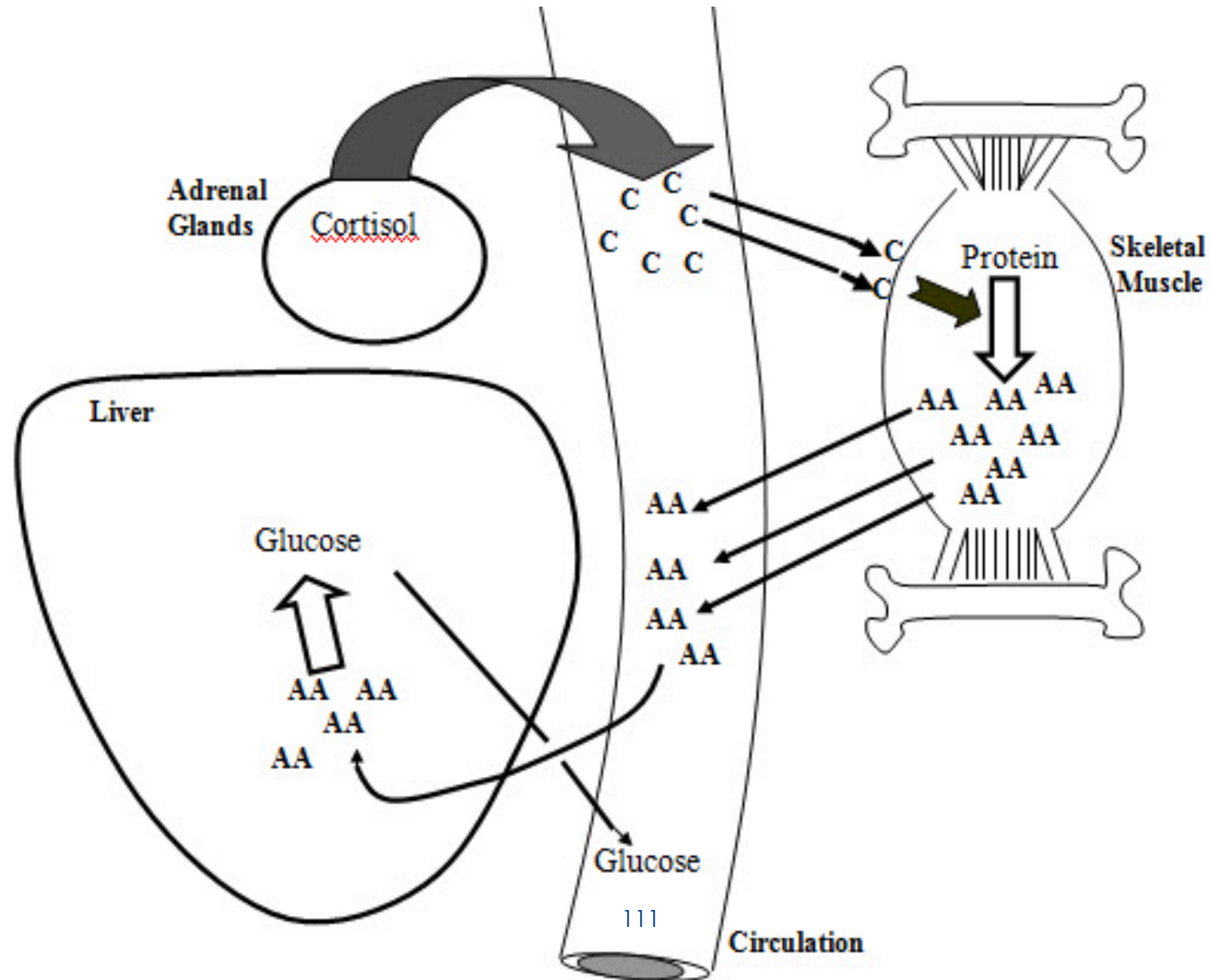
- * BOTH appear to negatively impact beta cell function
- * Excess ROS production is a major theory behind glucose toxicity
 - * Oxidative phosphorylation during anaerobic glycolysis
 - * Glycation
 - * Glucose autooxidation
- * High glucose levels increase intracellular peroxide levels and observed low levels of antioxidant enzymes
- * Lipotoxicity thought to be due to hyperlipidemia, especially of saturated long chain fatty acids
 - * Therapeutic ketogenic diet may be helpful at reducing toxicity (personal opinion)



Hyperthyroidism

- * Shorter half-life of insulin
- * increased intestinal glucose absorption
- * increased FFA and thus hepatic gluconeogenesis
- * excess lactate leading to increased hepatic gluconeogenesis (Cori cycle)
- * increase in GH, glucagon and catecholamines - all promote hyperglycemia

Hyper Adrenal Function (cortisol)



Glucose – Elevated

Cause	Reason	Additional Inquiry
Insulin resistance (increased glucagon)	Dysfunctional insulin signaling (eg receptors) causing glucose to remain elevated in the blood.	Fatigue after meals, sweets cravings even after carbohydrate ingestion; evaluate c-peptide, hemoglobin A1C
Pancreatic dysfunction	Poor insulin production	Evaluate c-peptide.
Hyperthyroidism	Shorter half-life of insulin, increased intestinal glucose absorption, increased FFA and thus hepatic gluconeogenesis, excess lactate leading to increased hepatic gluconeogenesis (Cori cycle), increase in GH, glucagon and catecholamines all promote hyperglycemia	Evaluate thyroid markers.
Hyperadrenal function	Increased cortisol and/or catecholamine increase glucose levels	Consider evaluating cortisol levels.

Glucose - Decreased

Cause	Reason	Additional Inquiry
Hypoadrenal function	Low cortisol levels leading to an inability to maintain stable glucose levels	Evaluate Na and K on blood chemistry; evaluate cortisol levels
Hypopituitary	Low TSH, low ACTH and low GH can all lead to hypoglycemia.	
Hypothyroidism	Decreased intestinal glucose absorption, decreased gastric emptying, decreased gluconeogenesis and glycogenolysis, decreased cortisol response, decreased glucagon production, blunted growth hormone response	Evaluate thyroid markers
Vitamin B6 deficiency	Pyridoxine (B6) is required for glycogen phosphorylase, the first and rate limiting step in glycogenolysis	Evaluate low AST and/or ALT levels; diet journal for B6 intake
Carnitine deficiency	See above – carnitine is important for the initial step of gluconeogenesis (pyruvate carboxylase)	Consider lysine and or methionine deficiency (key amino acid in carnitine synthesis, often deficiency in vegetarians), poor carnitine intake, or other deficiencies (eg vitamin C)

Glucose - Decreased

Vitamin C deficiency	Vitamin C is required for synthesis of carnitine, which is required to bring long-chain fatty acids into the mitochondria; high levels of Acetyl CoA in the mitochondria stimulate gluconeogenesis. Thus low carnitine -> low acetyl CoA -> poor gluconeogenesis -> low glucose.	Evaluate low alkaline phosphatase; bleeding gums; easy bruising
H. Pylori infection	There is growing evidence between H.Pylori and insulin resistance. It may be that reactive hypoglycemia may precede insulin resistance and have an H. Pylori connection.	
Biotin deficiency	Biotin is required for the initial enzyme of gluconeogenesis, pyruvate carboxylase and thus, may lead to hypoglycemia. On the other hand, it must be noted that biotin has other roles in glucose metabolism including the synthesis of glucokinase, PFK-1, and in allowing the pancreas to sense glucose and thus produce insulin.	
High-dose vitamin C	Vitamin C, specifically dehydroascorbic acid, competes with glucose across cell membranes and thus may slow intestinal glucose absorption. (**Conversely, high serum glucose may cause a relative vitamin C deficiency within cells.)	

Hypo Adrenal Function

- * Cortisol helps maintain glucose levels (glucocorticoid)
- * Thus if cortisol is low, glucose will often be low as well

Decreased Pituitary function

- * The anterior pituitary releases three hormones that could relate to glucose levels
 - * TSH – Thyroid
 - * ACTH – Cortisol
 - * Growth Hormone
- * If the pituitary is dysfunctional, all three of these hormones may be low, thus contributing to periods of low blood sugar
- * WHAT SUPPRESSES PITUITARY FUNCTION?

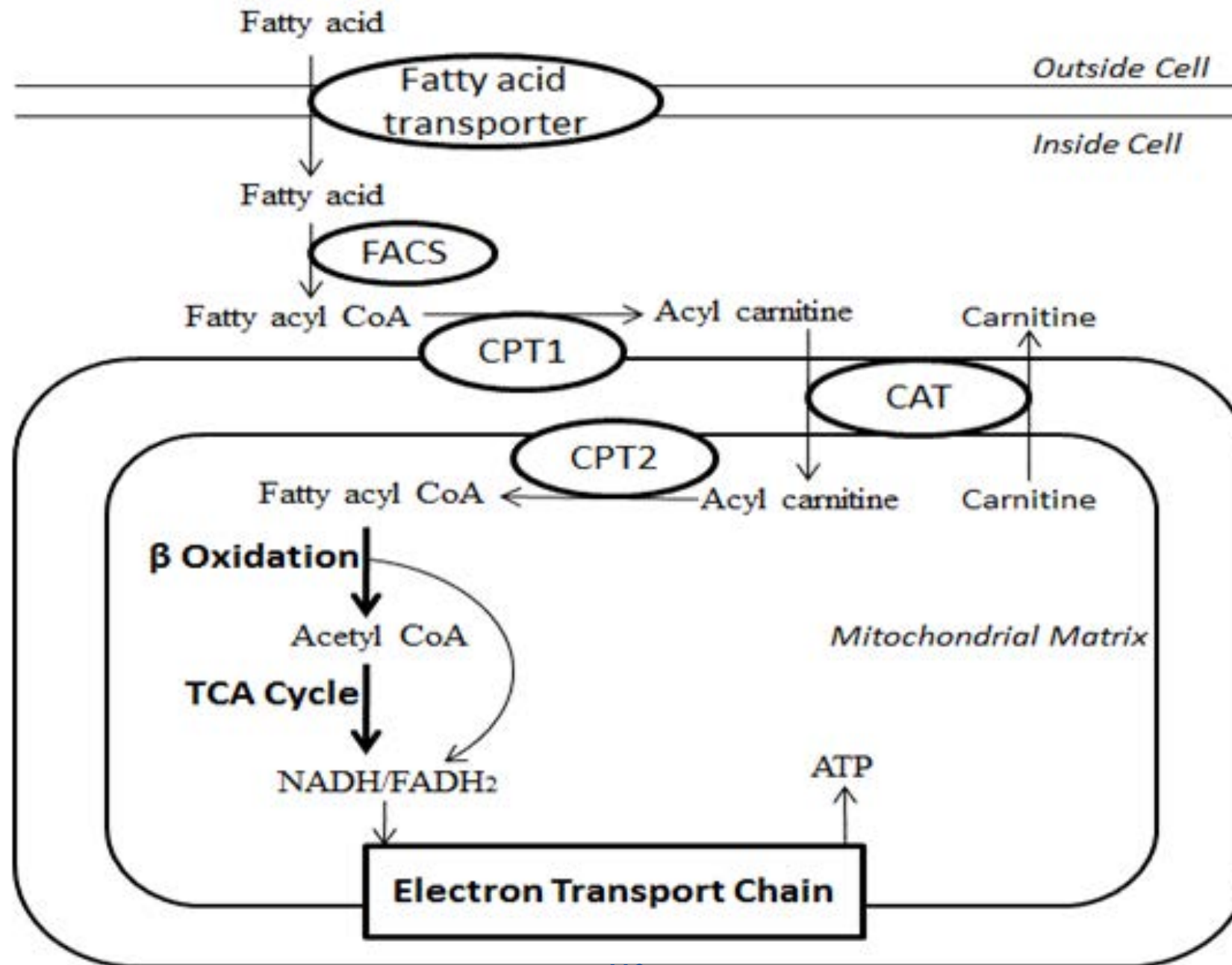
Hypothyroidism

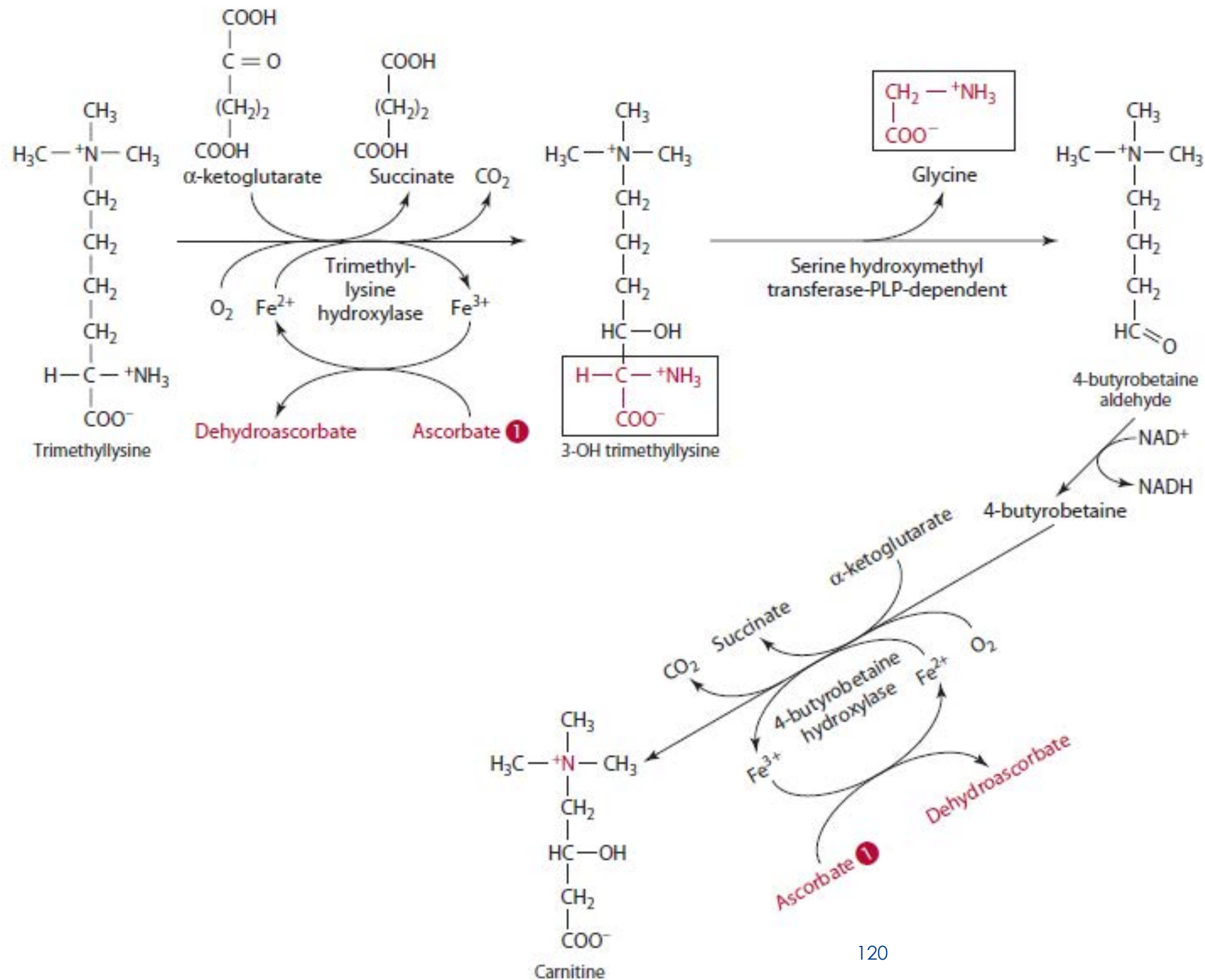
- * Decreased intestinal glucose absorption
- * Decreased gastric emptying
- * Decreased gluconeogenesis and glycogenolysis
- * Decreased cortisol response
- * Decreased glucagon production
- * Blunted GH response

Vitamin B6 (pyridoxine) deficiency

- * Glycogen phosphorylase – the rate limiting enzyme of glycogenolysis – is a B6 dependent enzyme
- * ***Low B6 may lead to hypoglycemic tendencies

Carnitine and/or vitamin C deficiency





Nutritional requirements for carnitine synthesis:

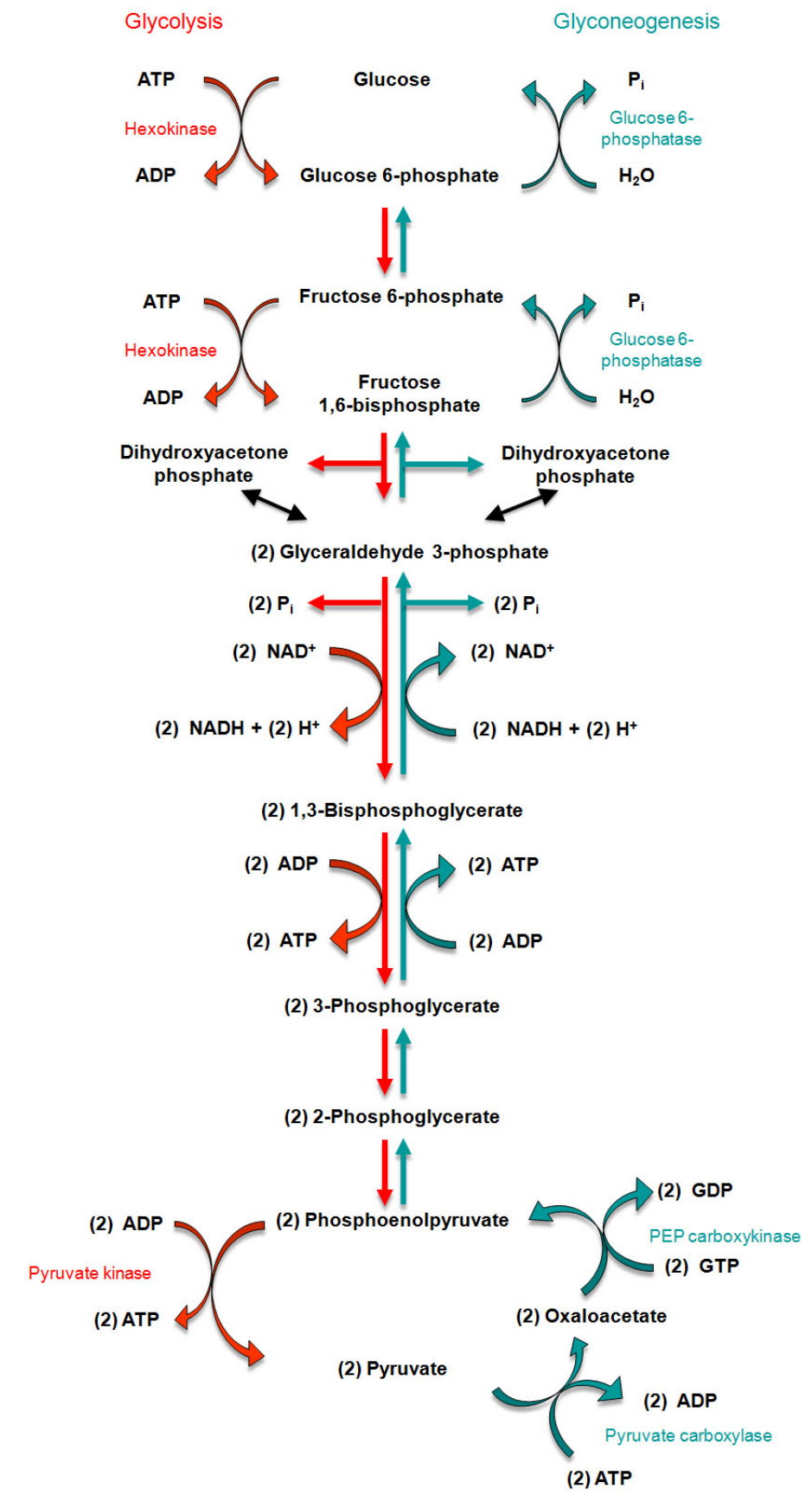
- Lysine
- Vitamin C
- Iron
- Oxygen
- Vitamin B6

H. Pylori Infection

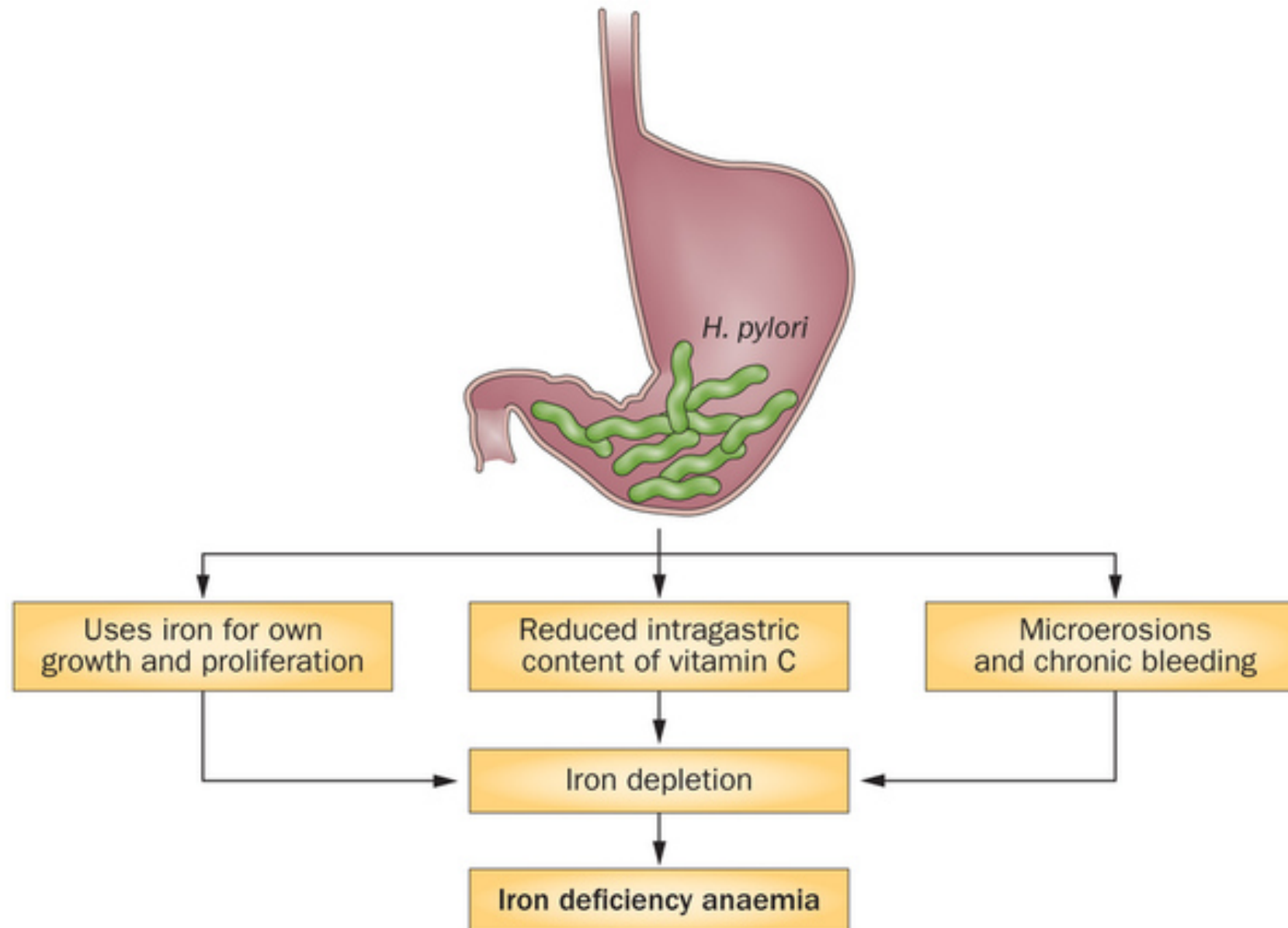
- * There is growing evidence between H.Pylori and insulin resistance.
- * ***If reactive hypoglycemia precedes insulin resistance, then it is possible that hypoglycemic symptoms may be caused in part by a concomitant H. pylori infection.

Biotin deficiency

- * Biotin is required (COFACTOR) for the initial enzyme of gluconeogenesis, pyruvate carboxylase and thus, may lead to hypoglycemia. On the other hand, it must be noted that biotin has other roles in glucose metabolism including the synthesis of glucokinase, PFK-1, and in allowing the **pancreas to sense glucose and thus produce insulin.**



H. Pylori/ Carnitine connection?



Glucose - Decreased

Cause	Reason	Additional Inquiry
Hypoadrenal function	Low cortisol levels leading to an inability to maintain stable glucose levels	Evaluate Na and K on blood chemistry; evaluate cortisol levels
Hypopituitary	Low TSH, low ACTH and low GH can all lead to hypoglycemia.	
Hypothyroidism	Decreased intestinal glucose absorption, decreased gastric emptying, decreased gluconeogenesis and glycogenolysis, decreased cortisol response, decreased glucagon production, blunted growth hormone response,	Evaluate thyroid markers
Vitamin B6 deficiency	Pyridoxine (B6) is required for glycogen phosphorylase, the first and rate limiting step in glycogenolysis	Evaluate low AST and/or ALT levels; diet journal for B6 intake
Carnitine deficiency	See above – carnitine is important for the initial step of gluconeogenesis (pyruvate carboxylase)	Consider lysine and or methionine deficiency (key amino acid in carnitine synthesis, often deficiency in vegetarians), poor carnitine intake, or other deficiencies

Glucose - Decreased

Vitamin C deficiency	Vitamin C is required for synthesis of carnitine, which is required to bring long-chain fatty acids into the mitochondria; high levels of Acetyl CoA in the mitochondria stimulate gluconeogenesis. Thus low carnitine -> low acetyl CoA -> poor gluconeogenesis -> low glucose.	Evaluate low alkaline phosphatase; bleeding gums; easy bruising
H. Pylori infection	There is growing evidence between H.Pylori and insulin resistance. It may be that reactive hypoglycemia may precede insulin resistance and have an H. Pylori connection.	
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High-dose vitamin C	Vitamin C, specifically dehydroascorbic acid, competes with glucose across cell membranes and thus may slow intestinal glucose absorption. (Conversely, high serum glucose may cause a relative vitamin C deficiency within cells.) ¹²⁵	

Glucose – Interfering Factors

Interfering Factors	
Elevated	Decreased
Non-fasting	Stress response before test
Medications - Corticosteroids	Medications
	Intense exercise

Reactive Hypoglycemia - Symptoms

- * Shaky, light-headed, irritable, especially between meals
- * May crave salt
- * Craves sweets, but corrected after sweets are consumed
- * Food provides energy
- * Consistently wakes up during the night

Reactive Hypoglycemia

- * Periods of low glucose causing primarily neurological symptoms
- * Could be due to excess insulin
- * Counter regulatory hormones (glucagon and cortisol) may not be working correctly
 - * Often find low cortisol in these individuals
- * The question is, why are they not able to maintain healthy glucose levels – glycogenolysis and gluconeogenesis
- * Due to increased GLP-1? (ENTEROENDOCRINE)

Reactive Hypoglycemia

- * Glycogenolysis requires vitamin B6
- * Low insulin levels should simulate free fatty acid release for use in beta-oxidation within mitochondria - to increase ATP
 - * Requires carnitine to shuttle long chain fatty acids into mitochondria
 - * Without carnitine fatty acids are not brought into the mitochondria
 - * When acetyl CoA levels are elevated (eg beta-oxidation), pyruvate carboxylase (biotin dependent enzyme) is upregulated, and gluconeogenesis proceeds. When acetyl CoA is low, pyruvate carboxylase is inhibited, and gluconeogenesis decreases.
- * Therefore, for hypoglycemia, consider B6, biotin, carnitine and vitamin C (required for carnitine synthesis)

Reactive Hypoglycemia – The Short Version

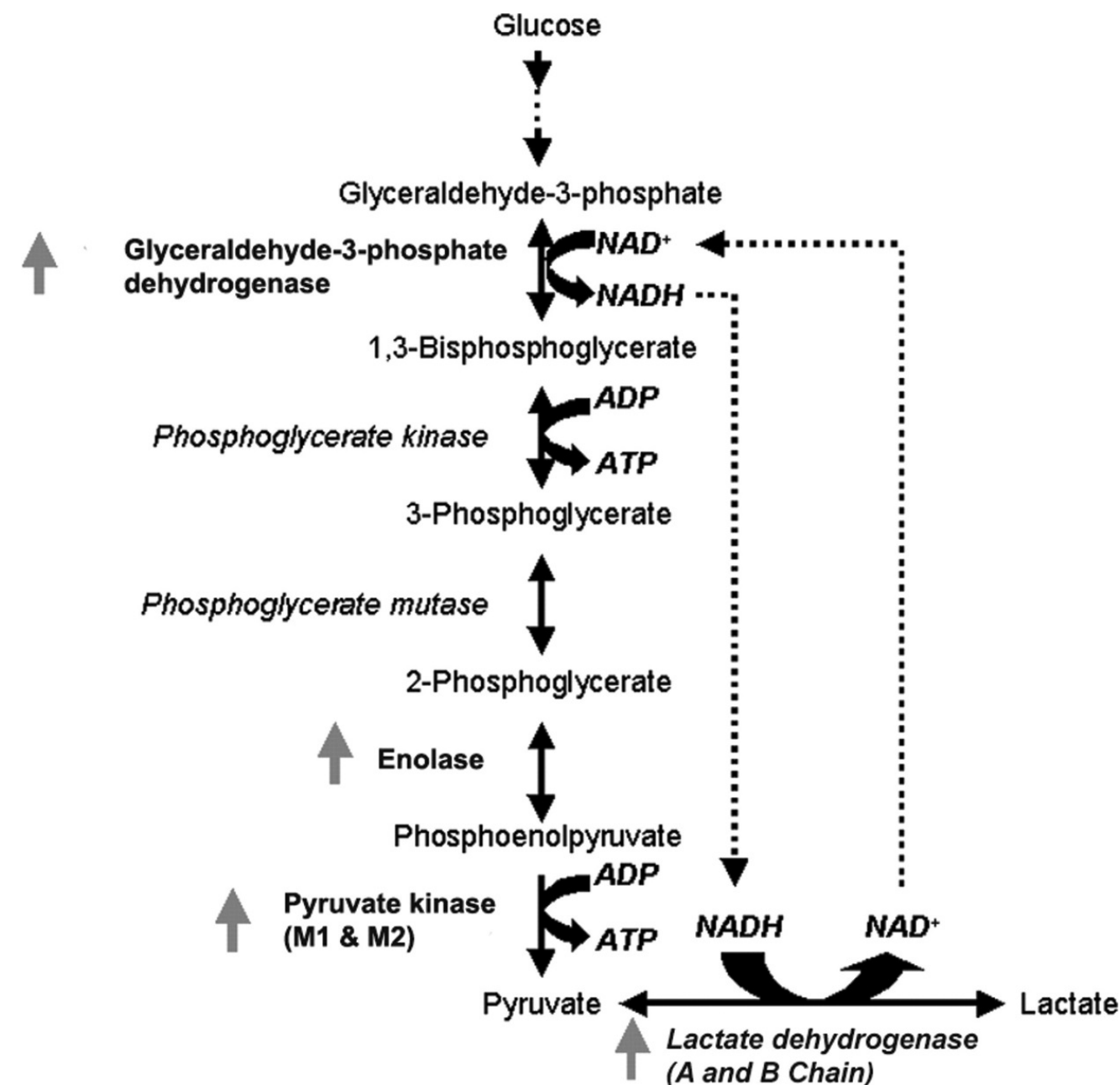
- * Hyperinsulinemia
- * Excess GLP-1 production
- * Low counter regulatory hormones
- * Glucagon resistance
- * Carnitine deficiency

Lactate Dehydrogenase (LDH)

- * Lactate Dehydrogenase (LDH) is an widely-distributed, intracellular enzyme found in skeletal and cardiac muscle, kidney, liver, lungs, and red blood cells.
- * As an intracellular enzyme, when LDH is elevated on a blood chemistry it suggests tissue damage. But due to its wide distribution in tissues, by itself is impossible to know where the damage is located.
- * LDH isoenzymes can better pinpoint the type of tissue being damaged

Lactate Dehydrogenase (LDH)

- * There are many possible reasons for elevated LDH, but low LDH can indicate poor glucose metabolism, aka hypoglycemic tendencies



Lactate Dehydrogenase (LDH)

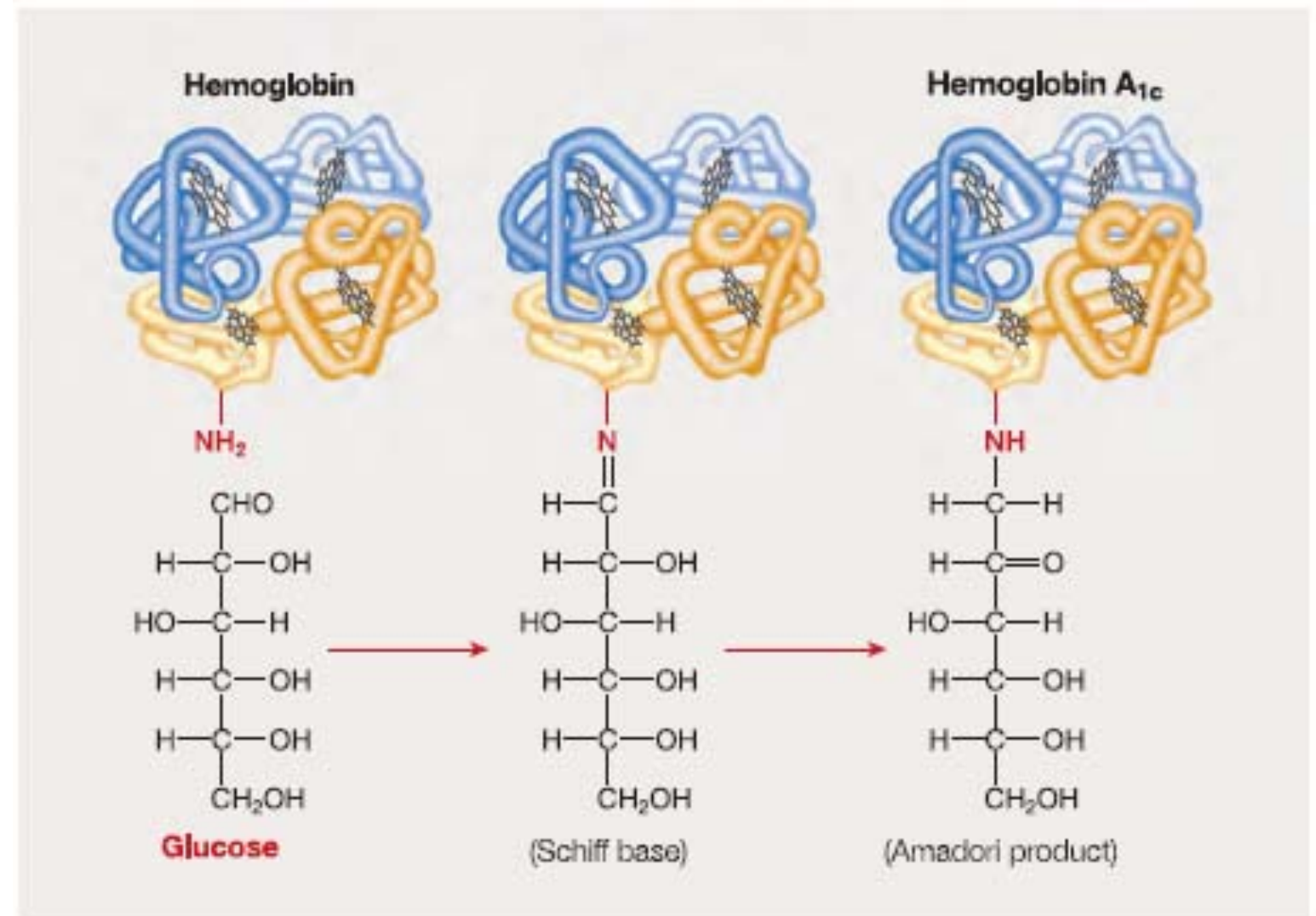
- * Traditional Reference range:
 - * 100–280 U/L
- * Optimal Reference Range:
 - * 140–170 U/L

LDH - Decreased

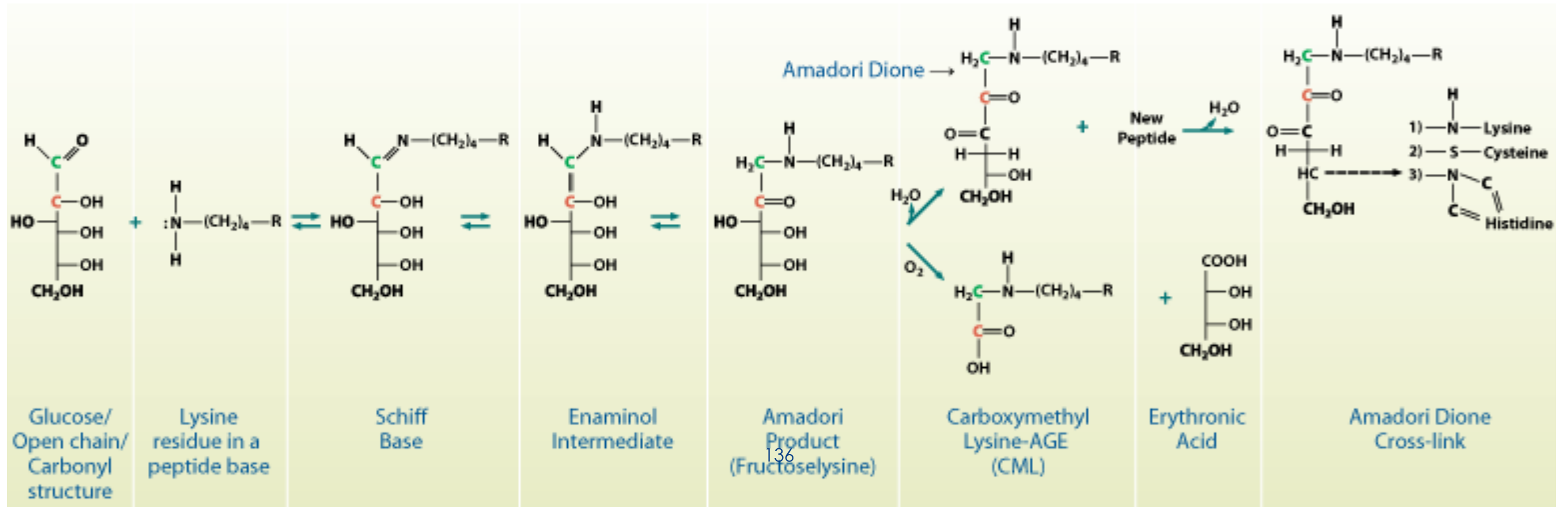
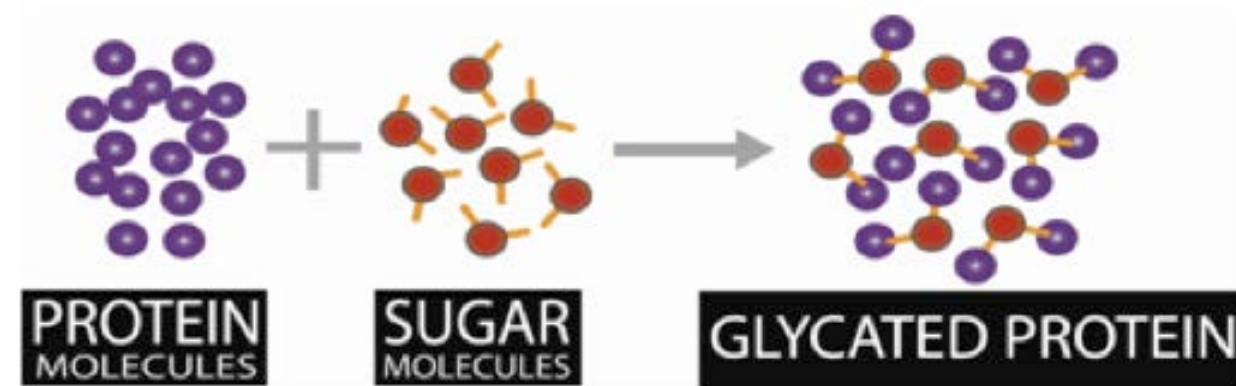
Cause	Reason	Additional Inquiry
Hypoglycemia	Poor glucose entry into a cell downregulates LDH enzyme production intracellularly, thus leading to low levels of LDH. (NADH/NAD ratio?)	Evaluate glucose levels and hypoglycemic symptoms.
Diabetes	Poor glucose entry into cells can decrease LDH production leading to low levels.	Evaluate hyperglycemic markers and symptoms.
Ketosis	Due to the utilization of ketones over glucose molecules during ketosis, it is possible someone following a ketogenic diet will have low LDH levels.	Client history. Presence of ketones.

Hemoglobin A1C

- * Glycated hemoglobin is formed at a rate proportional to the average glucose concentration by a slow, non-enzymatic process within red blood cells during their 120 day lifespan.
- * Glycated hemoglobin levels reflect blood sugar during a 2-3 month time period prior to the test.



Glycation



Hemoglobin A1C

Non-diabetic conditions	
Elevated	Decreased
Iron-deficiency anemia	Hemolytic anemia
Lead toxicity	Chronic blood loss
Long-lived RBC	Short-lived RBC

Hemoglobin A_{1c} Is Associated With Increased Risk of Incident Coronary Heart Disease Among Apparently Healthy, Nondiabetic Men and Women

	Quintile of HbA _{1c} *					P Trend [†]
	1	2	3	4	5	
Nurses' Health Study (371 cases/832 controls)						
HbA _{1c} , %	<5.1	5.1 to <5.3	5.3 to <5.5	5.5 to <5.7	≥5.7	
Cases/controls	62/166	61/168	75/165	84/167	89/166	
Model 1 [‡]	1.0	1.06 (0.70 to 1.62)	1.43 (0.94 to 2.18)	1.73 (1.12 to 2.66)	2.01 (1.26 to 3.21)	<0.001
Model 2 [§]	1.0	1.06 (0.69 to 1.65)	1.39 (0.89 to 2.16)	1.59 (1.01 to 2.50)	1.78 (1.09 to 2.91)	0.009
Model 3 [¶]	1.0	1.05 (0.68 to 1.63)	1.35 (0.86 to 2.10)	1.55 (0.98 to 2.44)	1.71 (1.04 to 2.80)	0.01
Model 4	1.0	1.05 (0.67 to 1.65)	1.39 (0.88 to 2.18)	1.52 (0.96 to 2.43)	1.70 (1.03 to 2.81)	0.02
Health Professionals Follow-Up Study (396 cases/843 controls)						
HbA _{1c} , %	<5.2	5.2 to <5.4	5.4 to <5.6	5.6 to <5.8	≥5.8	
Cases/controls	64/168	66/169	87/168	73/170	106/168	
Model 1 [‡]	1.0	1.12 (0.73 to 1.70)	1.56 (1.03 to 2.38)	1.35 (0.87 to 2.11)	1.97 (1.28 to 3.03)	0.001
Model 2 [§]	1.0	1.10 (0.71 to 1.68)	1.52 (0.99 to 2.33)	1.19 (0.75 to 1.88)	1.67 (1.07 to 2.59)	0.03
Model 3 [¶]	1.0	1.10 (0.72 to 1.69)	1.55 (1.01 to 2.39)	1.19 (0.75 to 1.88)	1.69 (1.08 to 2.64)	0.02
Model 4	1.0	1.08 (0.70 to 1.66)	1.57 (1.02 to 2.42)	1.17 (0.74 to 1.86)	1.69 (1.08 to 2.65)	0.02
Pooled						
Cases/controls	126/334	127/337	162/333	157/337	195/334	
Model 3 [¶]	1.0	1.08 (0.79 to 1.46)	1.45 (1.06 to 1.97)	1.36 (0.98 to 1.88)	1.70 (1.22 to 2.36)	<0.001

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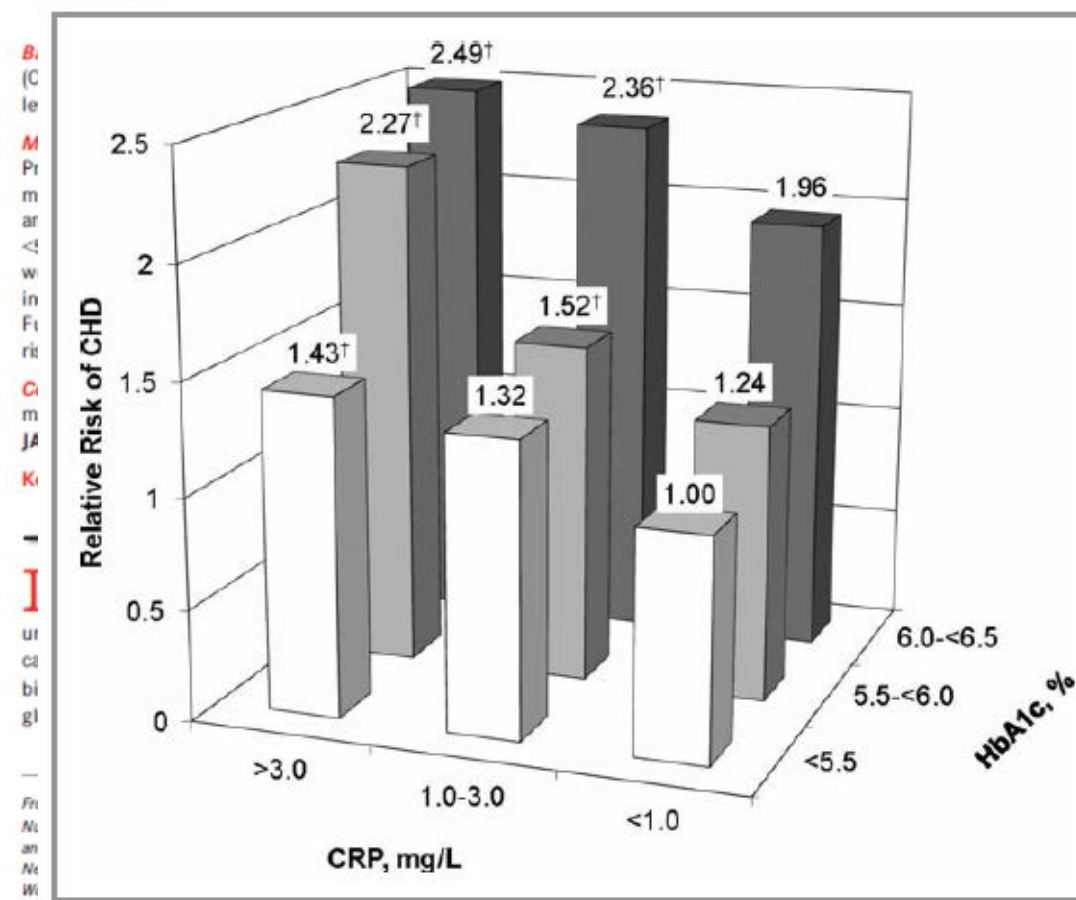
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between HbA_{1c} and CHD is attenuated and no longer significant after adjustment for well-known cardiovascular risk factors.^{11,13} Thus, it remains unclear whether HbA_{1c} is a clinically relevant marker of CHD risk in apparently healthy, nondiabetic populations, or if it is a proxy for dyslipidemia, hyperinsulinemia, or other metabolic pathways. In the present

Hemoglobin A_{1c} Is Associated With Increased Risk of Incident Coronary Heart Disease Among Apparently Healthy, Nondiabetic Men and Women

Jennifer K. Pai, ScD, MHS; Leah E. Cahill, PhD; Frank B. Hu, MD, PhD; Kathryn M. Rexrode, MD, MPH; JoAnn E. Manson, MD, DrPH; Eric B. Rimm, ScD



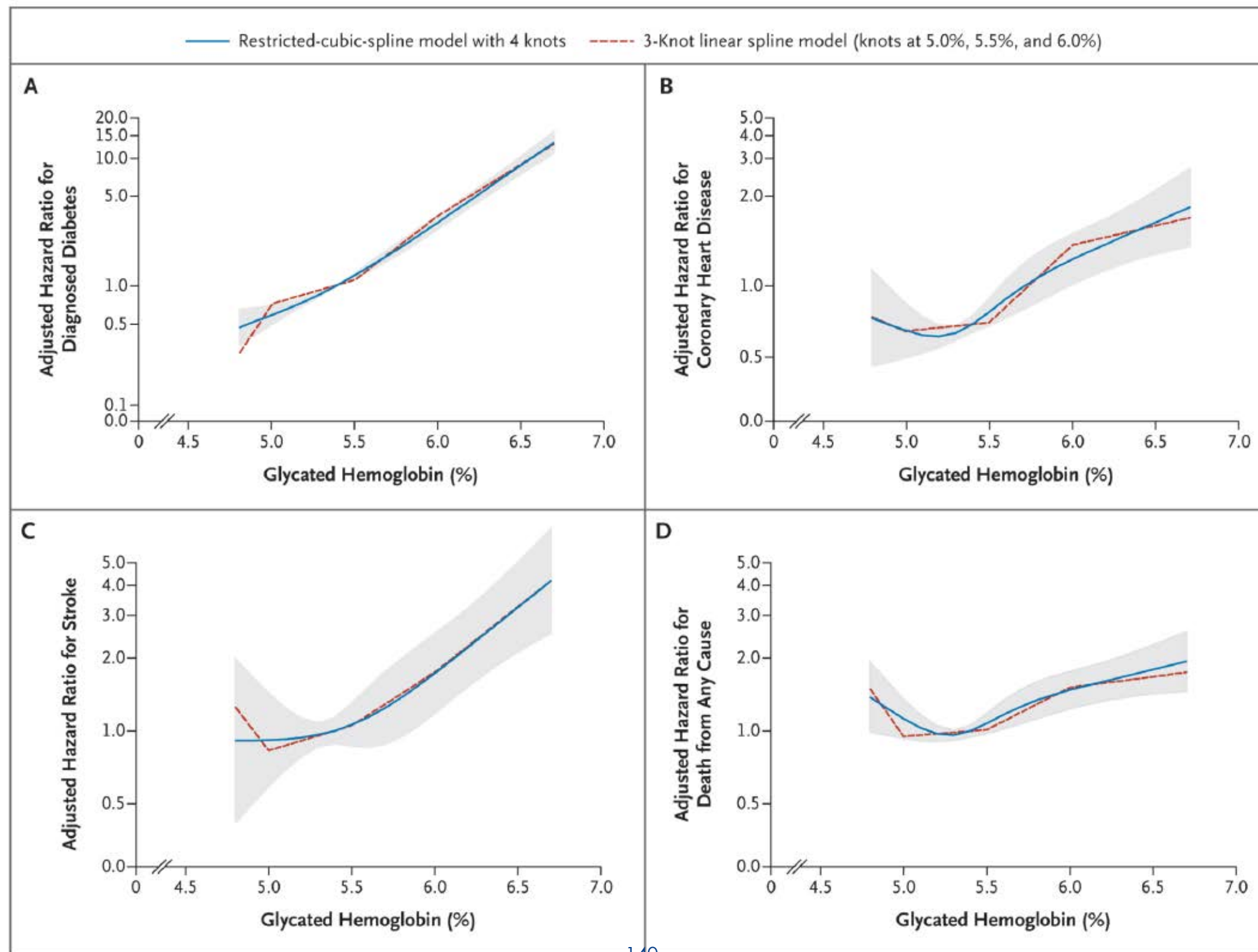
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with HbA_{1c} ≥6.5%,^{11,12} the threshold set for diabetes diagnosis by the American Diabetes Association² and World Health Organization.⁴ In several studies, the association between HbA_{1c} and CHD is attenuated and no longer significant after adjustment for well-known cardiovascular risk factors.^{11,13} Thus, it remains unclear whether HbA_{1c} is a clinically relevant marker of CHD risk in apparently healthy, nondiabetic populations, or if it is a proxy for dyslipidemia, hyperinsulinemia, or other metabolic pathways. In the present



RESEARCH ARTICLE

Open Access



HbA_{1c} levels in non-diabetic older adults – No J-shaped associations with primary cardiovascular events, cardiovascular and all-cause mortality after adjustment for confounders in a meta-analysis of individual participant data from six cohort studies

Ben Schöttker^{1,2*}, W. Rathmann³, C. Herder^{4,5}, B. Thorand⁶, T. Wilsgaard⁷, I. Njølstad⁷, G. Siganos⁸, E. B. Mathiesen⁸,
K. U. Saum¹, A. Peasey⁹, E. Feskens¹⁰, P. Boffetta^{11,12}, A. Trichopoulos¹², K. Kuulasmaa¹³, F. Kee¹⁴, H. Brenner¹
and on behalf of the CHANCES group

This investigation is a meta-analysis of individual participant data of six population-based cohort studies: Tromsø (Norway); ELSA (UK); NHANES (USA); ESTHER (Germany); KORA (Germany); and SHIP (Germany).

Results: Overall, 6,769 of 28,681 study participants died during a mean follow-up of 10.7 years, of whom 2,648 died of cardiovascular disease. Furthermore, 2,493 experienced a primary cardiovascular event. A linear association with primary cardiovascular events was observed. Adjustment for cardiovascular risk factors explained about 50 % of the excess risk and attenuated hazard ratios (95 % confidence interval) for increased HbA_{1c} to 1.14 (1.03–1.27), 1.17 (1.00–1.37) and 1.19 (1.04–1.37) for all-cause mortality, cardiovascular mortality and cardiovascular events, respectively. The six cohorts yielded inconsistent results for the association of very low HbA_{1c} levels with the mortality outcomes and the pooled effect estimates were not statistically significant. In one cohort with a pronounced J-shaped association of HbA_{1c} levels with all-cause and cardiovascular mortality (NHANES), the following confounders of the association of very low HbA_{1c} levels with mortality outcomes were identified: race/ethnicity; alcohol consumption; BMI; as well as biomarkers of iron deficiency anemia and liver function. Associations for very low HbA_{1c} levels lost statistical significance in this cohort after adjusting for these confounders.

(Continued on next page)

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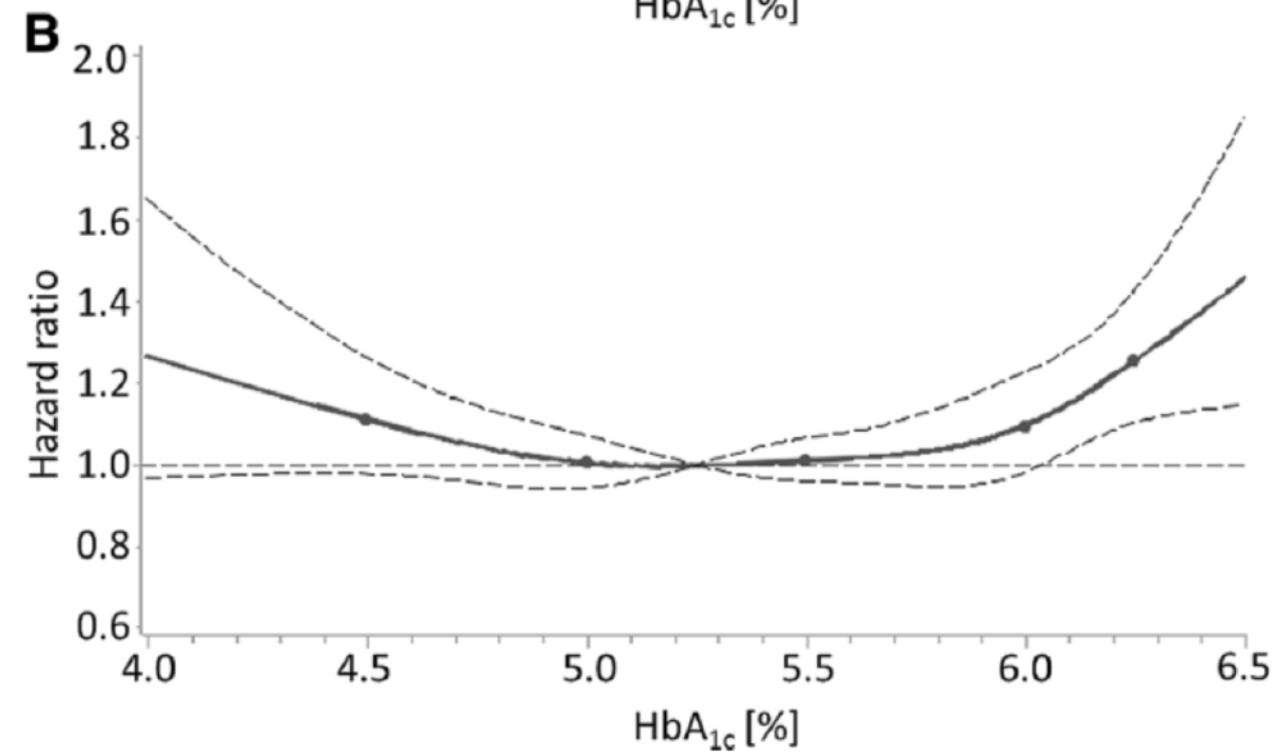
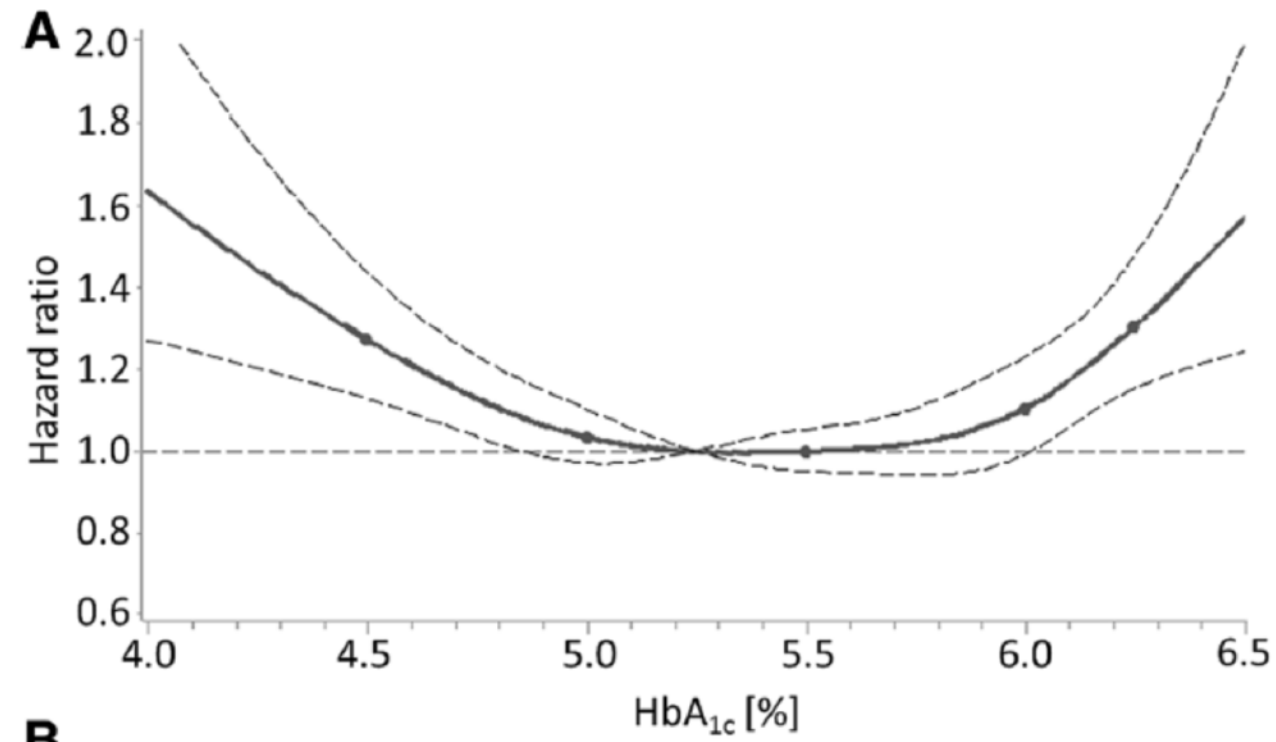
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HbA1C and Its Correlations

A number of studies suggest that an A1C above 5.6% is predictive of CAD and impaired glucose tolerance, even with normal fasting glucose.

Hemoglobin A1C

- * Optimal Reference Range:

- * 5.1-5.3%*

*If lower, likely not hyperglycemia or glucose dysregulation, but according to studies, does not seem optimal

HbA1C Limitations

- * Red blood cell life span
- * Unexplained variations between mean glucose and A1C among patients with diabetes (more pronounced at lower levels of A1C)
- * Does not discriminate between the patient with excessive fluctuations and chronically elevated glucose

Calculating RBC Lifespan

- * Red Blood Cell Survival (days) = $100 / [\text{reticulocytes (percent)} / \text{reticulocyte life span (days)}]$
- * Helpful in determining accuracy of hemoglobin A1C
 - * Longer-lived RBC, leads to more hemoglobin, and thus a falsely elevated HbA1C (false positive)
 - * Shorter-lived RBC, leads to less hemoglobin, and thus a falsely decreased HbA1C (false negative)

Calculating RBC Lifespan

Reticulocyte count: 0.8%

Hematocrit: 45

Correction of Reticulocyte Count	
Hematocrit (%)	Reticulocyte Life Span (RLS)
36-45	1.0
26-35	1.5
16-25	2.0
<15	2.5

Adapted from Harrisons Principles of Internal Medicine, 18th edition.

Thus, the equation would look like this:

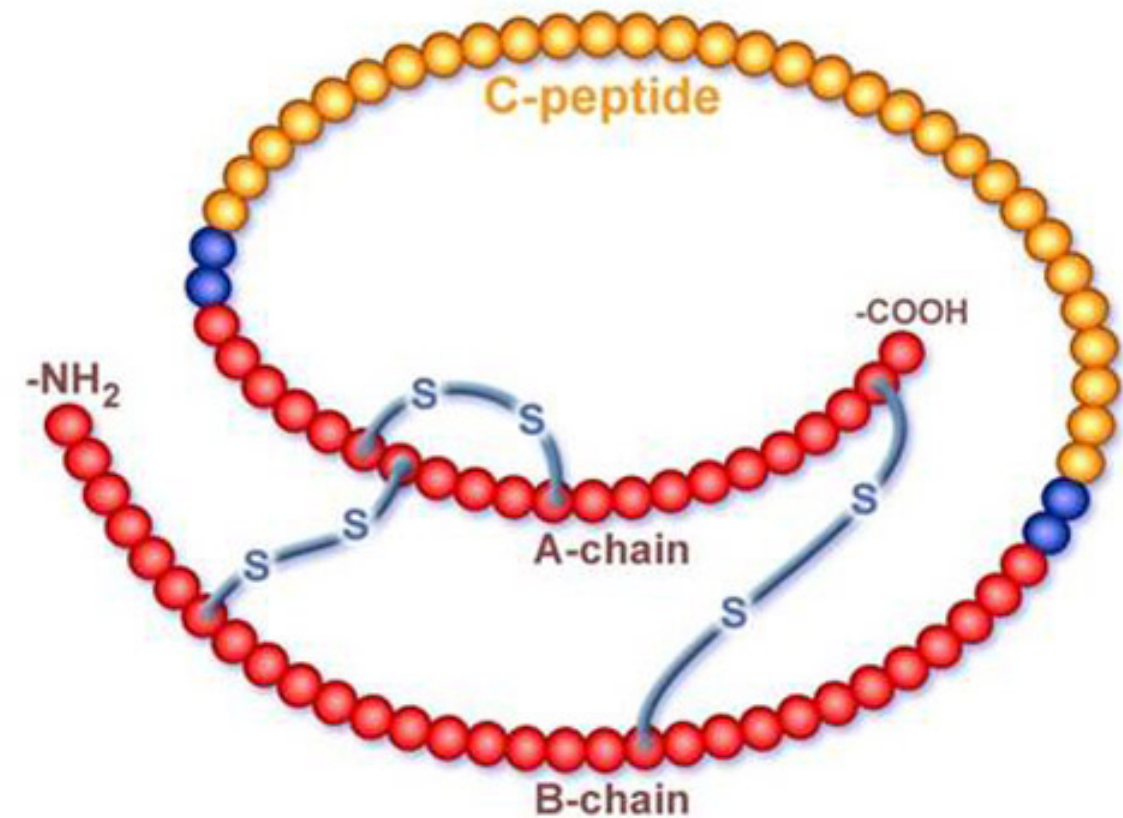
$$100/[0.8/1] = 125 \text{ days}$$

C-Peptide

Traditional Reference Range: 1.1-4.4 ng/mL

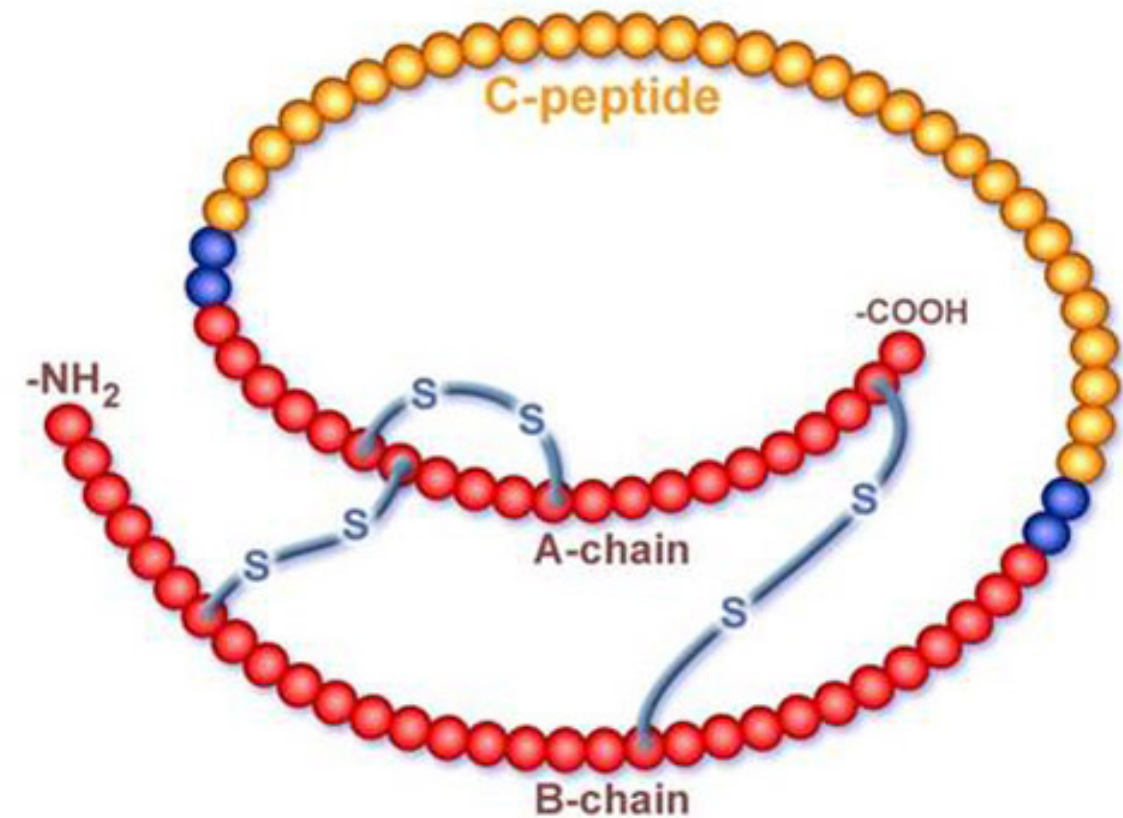
C-peptide

- * C-peptide is formed when proinsulin is converted to insulin.
- * C-peptide is released at the same time and in the same quantities as insulin, but has a longer half-life and is thus a more stable and reliable measurement than insulin.
- * May have its own metabolic activity, but more studies are needed



C-peptide

- * C-peptide is a more reliable indicator of insulin secretion
- * Not cleared by the liver
- * Has a longer half-life than insulin (30 minutes compared to **4 minutes for insulin**)
- * The pharmacokinetics of C-peptide have been well established in research studies.



Fasting Serum C-Peptide Levels Predict Cardiovascular and Overall Death in Nondiabetic Adults

Nileshkumar Patel, MD; Tracey H. Taveira, PharmD; Gaurav Choudhary, MD; Hilary Whitlatch, MD; Wen-Chih Wu, MD, MPH

Background—Insulin resistance, characterized by hyperinsulinemia and normal or elevated serum glucose, is an established precursor to diabetes and cardiovascular disease. Despite fasting serum C-peptide levels being an accurate and stable marker of endogenous insulin production used in patients with diabetes, it is unknown whether C-peptide could serve as a marker of insulin resistance and predict outcomes in patients without diabetes.

Method and Results—This is a retrospective cohort study using data from the NHANES-3 (1988–1994) survey with mortality follow-up through December 31, 2006. Participants included 5153 subjects, 40 to 74 years of age with fasting glucose ≥ 70 mg/dL, without diabetes by history or laboratory testing. Receiver-operating-curve analysis compared fasting C-peptide against known insulin resistance measures such as fasting plasma glucose, serum insulin, HOMA-IR, quantitative-insulin-sensitivity-check-index, and metabolic syndrome for the prediction of cardiovascular and overall death. Subjects were then stratified by quartiles of C-peptide levels. Cox proportional-hazards modeling compared hazards of cardiovascular and overall death amongst C-peptide quartiles and adjusted for potential confounders of cardiovascular and diabetes risk. Fasting serum C-peptide levels predicted cardiovascular and overall death better than other studied measures (AUC=0.62 and 0.60 respectively vs the rest, with AUC $\leq$ 0.58 and $\leq$ 0.57 respectively, $P<0.001$). When compared with the lowest C-peptide quartile, subjects in the highest quartile had

Downloaded from

C-peptide was superior to other insulin-derived measures of insulin resistance in predicting cardiovascular and overall death in nondiabetic adults. **C-peptide predicted cardiovascular death even in subjects with normoglycemia and without metabolic syndrome.** Prospective studies are needed to elucidate the usefulness of C-peptide levels as a target for intervention to improve cardiovascular outcomes.

These results would support that the increased risk of cardiovascular death in subjects with high C-peptide levels is unlikely to be attributed solely to the eventual development of diabetes, and suggest additional or alternative mechanisms of harm.¹⁵¹



C-Peptide Category, nmol/L						
	Overall	≤0.418	0.419 to 0.652	0.653 to 0.983	≥0.984	P Value
		1.26	1.26-1.96	1.96-2.95	>2.95	
No. of participants	5153	1288	1288	1290	1287	<0.01
Mean age±SD, y	54.9±10.5	53.4±10.2	55.0±10.5	55.1±10.4	56.1±10.7	
History of stroke, %	1.7	1.2	1.4	2.1	2.1	0.58
History of heart attack, %	4.0	2.4	2.7	4.3	7.1	<0.01
History of chest pain, %						<0.01
Suggestive of angina	4.7	3.3	3.8	5.3	7.0	
Not suggestive of angina	28.7	26.6	25.1	30.7	33.2	
No chest pain	66.6	70.1	71.1	64	59.8	
Peripheral arterial disease, %	0.9	0.2	1.3	0.3	2.3	<0.01
Mean hemoglobin A1c±SD, %	5.4±0.5	5.3±0.4	5.4±0.4	5.4±0.5	5.5±0.5	
Mean fasting plasma glucose±SD, mg/dL	95.5±9.2	90.9±7.5	94.8±8.2	96.8±8.8	99.5±9.8	<0.01
Mean 2 h OGTT±SD, mg/dL	121.2±34	115.6±34.8	117.2±32.9	124.4±33.4	127.2±33.3	<0.01
Mean serum cholesterol±SD, mg/dL	220.6±43.1	213.9±41.9	221.3±40.4	222.2±44.8	224.9±44.3	<0.01
Mean serum HDL±SD, mg/dL	51.5±16.2	59.6±17.5	53.4±15.9	48.4±13.9	44.7±13.2	<0.01
Mean serum triglycerides±SD, mg/dL	149.4±106.2	105.6±69.7	128.8±76.8	161.4±106.2	201.9±133.8	<0.01
Mean calculated serum LDL±D, mg/dL [†]	140.4±38.7	133.9±38.3	142.8±37.7	142.5±39.5	142.6±38.8	<0.01
Mean serum insulin±SD, pmol/L	65.2±47.3	34.4±12.7	47.6±13.4	64.7±19.1	114.2±67.8	<0.01
Mean serum CRP±SD, mg/dL [‡]	0.5±0.7	0.4±0.6	0.4±0.7	0.5±0.8	0.6±0.7	<0.01

C-Peptide Category, nmol/L					
	Overall	≤0.418	0.419 to 0.652	0.653 to 0.983	≥0.984
No. of participants	5140	1284	1283	1290	1283
Cardiovascular mortality, %*	7.2	3.8	6.0	7.9	12.2
Crude HR for cardiovascular mortality (95% CI)		Referent	1.61 (1.13 to 2.33)	2.18 (1.40 to 3.28)	3.44 (2.38 to 5.03)
Adjusted HR for cardiovascular mortality (95% CI)		Referent	1.20 (0.80 to 1.80)	1.42 (0.90 to 2.26)	1.60 (1.07 to 2.39)
No. of participants	5140	1284	1283	1290	1283
Cerebrovascular disease mortality, %*	1.0	0.5	0.6	0.8	2.2
Crude HR for cerebrovascular disease mortality (95% CI)		Referent	1.33 (0.53 to 3.37)	1.83 (0.60 to 5.57)	4.82 (1.81 to 12.8)
Adjusted HR for cerebrovascular disease mortality (95% CI)		Referent	1.12 (0.44 to 2.83)	1.22 (0.28 to 5.34)	3.94 (1.28 to 12.1)
No. of participants	5140	1284	1283	1290	1283
Ischemic heart disease mortality, %*	3.9	2.1	3.5	4.2	6.3
Crude HR for ischemic heart disease mortality (95% CI)		Referent	1.67 (1.04 to 2.69)	2.05 (1.30 to 3.26)	3.15 (2.07 to 4.81)
Adjusted HR for ischemic heart disease mortality (95% CI)		Referent	1.17 (0.66 to 2.10)	1.16 (0.65 to 2.06)	1.22 (0.67 to 2.21)
No. of participants	5140	1284	1283	1290	1283
Myocardial infarction mortality, %*	1.5	1.0	1.3	1.1	2.9
Crude HR for myocardial infarction mortality (95% CI)		Referent	1.22 (0.59 to 2.51)	1.13 (0.44 to 2.92)	3.00 (1.32 to 6.79)
Adjusted HR for myocardial infarction mortality (95% CI)		Referent	0.79 (0.37 to 1.67)	0.71 (0.25 to 2.01)	1.37 (0.66 to 2.83)
No. of participants	5152	1288	1287	1290	1287
Overall mortality, %*	18.1	12.3	14.7	20.6	26.4
Crude HR for overall mortality (95% CI)		Referent	1.22 (0.94 to 1.57)	1.77 (1.42 to 2.18)	2.31 (1.77 to 2.99)
Adjusted HR for overall mortality (95% CI)		Referent	1.12 (0.88 to 1.43)	1.57 (1.27 to 1.95)	1.72 (1.34 to 2.21)

Serum C-peptide levels and risk of death among adults without diabetes mellitus

Jin-young Min PhD, Kyoung-bok Min MD PhD

Competing interests: None declared.

This article has been peer reviewed.

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CMAJ 2013; DOI:10.1503/cmaj.121950

ABSTRACT

Background: Connecting peptide (C-peptide) plays a role in early atherogenesis in patients with diabetes mellitus and may be a marker for cardiovascular morbidity and mortality in patients without diabetes. We investigated whether serum C-peptide levels are associated with all-cause, cardiovascular-related and coronary artery disease-related mortality in adults without diabetes.

Methods: We used data from the Third Nutrition and Health Examination Survey (NHANES

cardiovascular-related and coronary artery disease-related mortality.

Results: The mean serum C-peptide level in the study sample was 0.78 ($\pm$ standard deviation 0.47) nmol/L. The adjusted hazards ratio comparing the highest quartile with the lowest quartile was 1.80 (95% confidence interval [CI] 1.33–2.43) for all-cause mortality, 3.20 (95% CI 2.07–4.93) for cardiovascular-related mortality, and 2.73 (95% CI 1.55–4.82) for coronary artery disease-related mortality. Higher C-peptide levels were associ-

We found an association between serum C-peptide levels and all-cause and cause-specific mortality among adults without diabetes at baseline. Our finding suggests that elevated C-peptide levels may be a predictor of death.

erable amount of insulin is extracted by the liver, C-peptide is subjected to negligible first-pass metabolism by the liver, thereby serving as a surrogate marker for endogenous insulin secretion.² C-peptide has been considered an inert by-product of insulin synthesis and has also been of great value in the understanding of the pathophysiology of type 1 and type 2 diabetes mellitus.^{2,3} However, C-peptide has recently been re-evaluated as a bioactive peptide in its own right. The administration of C-peptide to patients and animals with type 1 diabetes has been reported to have a beneficial effect on diabetes-induced abnormalities of the peripheral nerves and renal and microvascular function.^{4,5} C-peptide deposition occurs in the atherosclerotic lesions of patients with diabetes.⁶ Recent studies have suggested that C-peptide may be a valuable predictor of cardiovascular events and mortality (all-cause and cardiovascular-related mortality).^{6–12}

diabetes. We also estimated mortality as C-peptide increased across glycated hemoglobin and fasting blood glucose quartiles.

Methods

Study population

We used data from the Third National Health and Nutrition Examination Survey (NHANES III, 1988–94) database¹³ and the NHANES III Linked Mortality Public-use File from the United States. The latter was a follow-up study of mortality that matched records from NHANES III with data in the National Death Index as of Dec. 31, 2006. NHANES III included 39 695 participants aged 2 months and older, with a weighted response rate of 82% for household interviews and 73% for examinations.¹⁴ The date and cause of death in the National Death Index were derived from death certificates.¹⁵

Serum C-peptide levels and risk of death among adults without diabetes mellitus

Jin-young Min PhD, Kyoung-bok Min MD PhD

Competing interests: None declared.

This article has been peer reviewed.

ABSTRACT

Background: Connecting peptide (C-peptide) plays a role in early atherogenesis in patients with diabetes mellitus and may be a marker of cardiovascular-related and coronary artery disease-related mortality.

Participants who had high serum C-peptide levels (> 1.018 nmol/L) had a 1.8- to 3.2-fold increased risk of all-cause and cause-specific mortality compared with those who had low C-peptide levels (< 0.440 nmol/L). This risk was progressive: after adjustment, each 1-unit increase in baseline C-peptide level was associated with 41%–70% increases in the relative risk of death.

We found that C-peptide level better predicted all-cause, cardiovascular-related and coronary artery disease-related mortality than did glycated hemoglobin or fasting blood glucose level.

< 0.440 , 0.440 – 0.692 , 0.693 – 1.018 , > 1.018 nmol/L

< 1.32 , 1.32 – 2.078 , 2.081 – 3.06 , > 3.06 ng/mL

Fasting C-Peptide Levels and Death Resulting From All Causes and Breast Cancer: The Health, Eating, Activity, and Lifestyle Study

Melinda L. Irwin, Catherine Duggan, Ching-Yun Wang, Ashley Wilder Smith, Anne McTiernan, Richard N. Baumgartner, Kathy B. Baumgartner, Leslie Bernstein, and Rachel Ballard-Barbash

See accompanying articles on pages 32, 40, and 54 and editorial on page 7

Among women without type 2 diabetes, **fasting C-peptide levels were associated with an increased risk of death resulting from all causes and from breast cancer.**

Table 3. Fasting C-Peptide and Risk of Death Resulting from Breast Cancer in Women Diagnosed With Breast Cancer

C-Peptide Level	No. of Events	Total No. of Patients	Age-Adjusted Model		Age and BMI-Adjusted Model		Multivariable-Adjusted Model*	
			HR	95% CI	HR	95% CI	HR	95% CI
C-peptide, ng/mL†								
< 1.7	7	184	1.00		1.00		1.00	
1.7-2.5	11	187	1.70	0.65 to 4.44	1.96	0.74 to 5.22	2.19	0.74 to 6.47
> 2.5	10	175	1.71	0.64 to 4.58	2.70	0.97 to 7.95	2.39	1.00 to 7.52
P for trend				.28		.072		.050
C-peptide, ng/mL								
< 1.7	7	184	1.00		1.00		1.00	
1.7-2.5	11	187	1.69	0.65 to 4.41	1.92	0.72 to 5.07	2.19	0.75 to 6.42
> 2.5	10	175	1.70	0.64 to 4.54	2.67	0.98 to 7.68	2.55	0.82 to 7.89
Women with type 2 diabetes	5	58	2.81	0.86 to 9.23	4.69	1.30 to 16.91	2.83	1.03 to 11.69
C-peptide, ng/mL (continuous)‡	28	546	1.21	0.92 to 1.58	1.37	1.04 to 1.82	1.35	1.02 to 1.87
P				.18		.027		.048

Women with type 2 diabetes. A recent publication from the Women's Health Initiative Observational Study showed that insulin levels were

independently associated with an increased risk of breast cancer death in women with high versus low fasting insulin levels.

WHY C-peptide and cancer?

Higher c-peptide levels were associated with faster decline in global cognition and verbal memory. We found that each year of age was associated with a greater annual rate of decline by -0.01 units on the global score; thus, our finding of greater annual decline of -0.03 units comparing the highest to lowest c-peptide quartile would indicate that higher levels of c-peptide are cognitively equivalent to 3 years of aging.

Q1 = 1.08

Q2 = 1.56

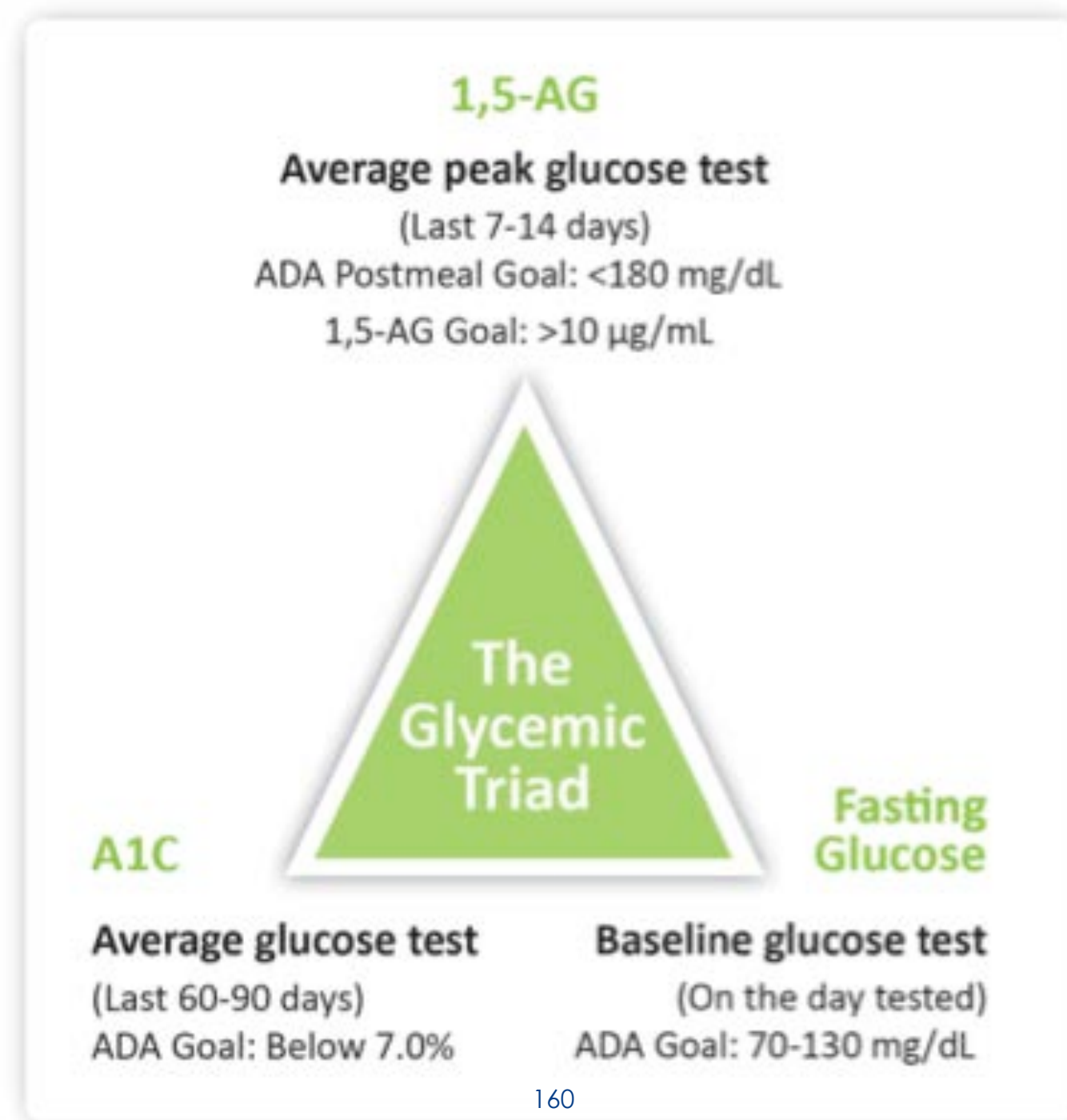
Q3 = 2.07

Q4 = 3.3

C-peptide

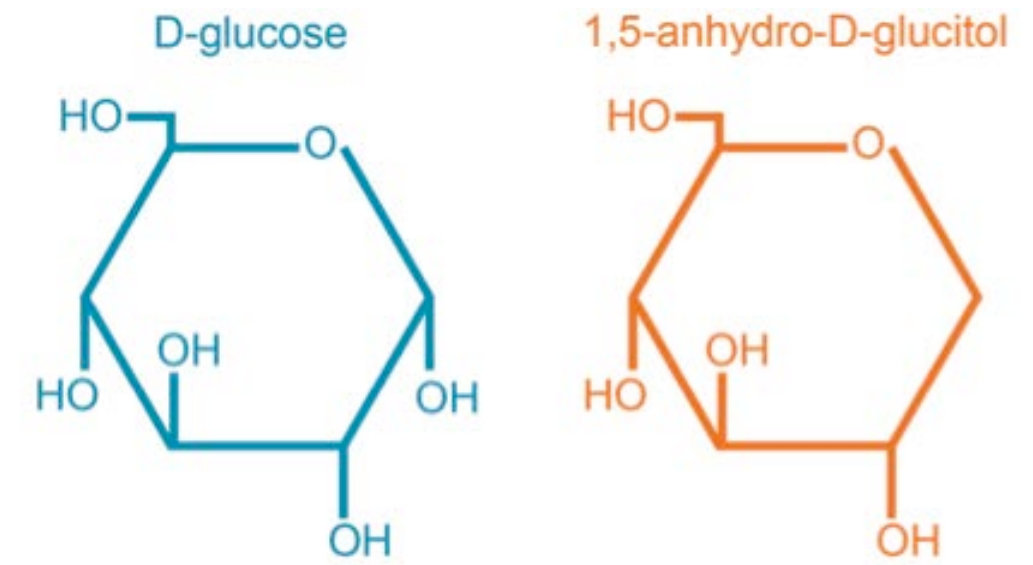
- * Traditional Range: 1.1 - 4.4 ng/mL
- * Optimal Range: 1.1 - 2.3 ng/mL (1.1-1.9?)

Glycomark



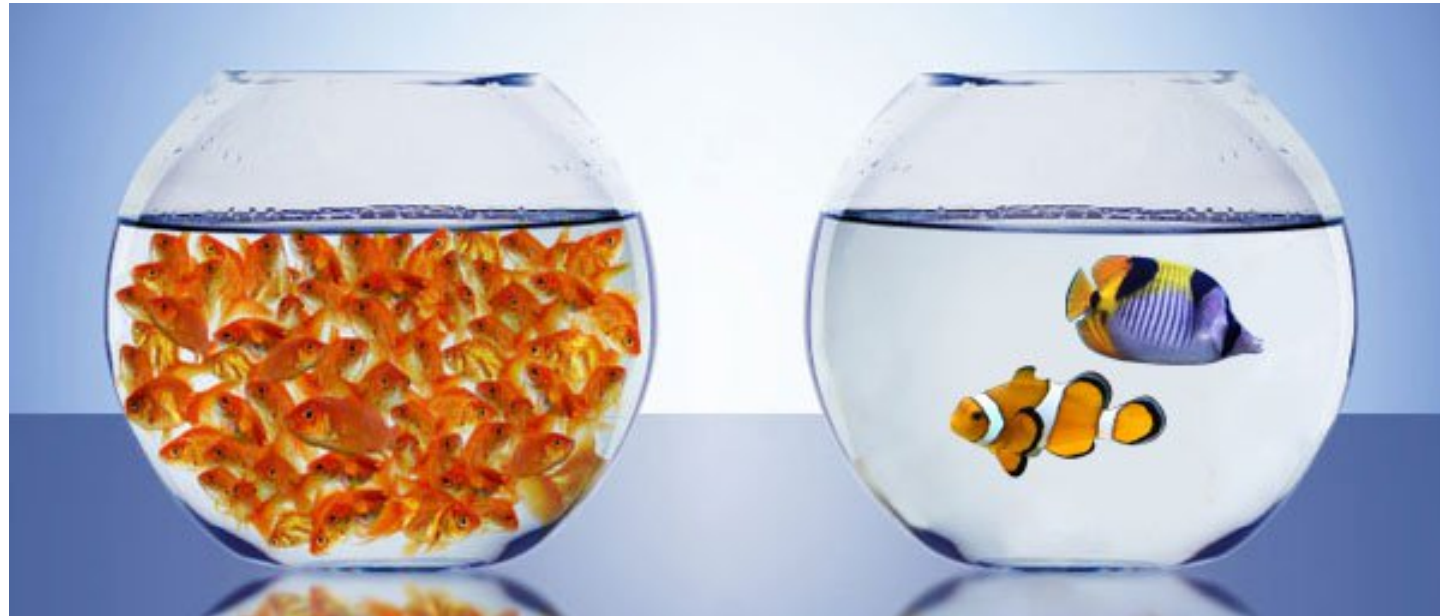
GlycoMark

- * 1,5-Anhydroglucitol (1,5-AG)
 - * Naturally occurring monosaccharide in food
 - * Not metabolized or used for biochemical processes
 - * Normally filtered and re-absorbed in by the kidneys under normoglycemic conditions
 - * However, when glucose levels are elevated, glucose and 1,5-AG compete for re-absorption in the kidneys and ultimately 1,5-AG loses and is excreted in urine *leading to lower serum levels*
 - * Occurs when serum glucose levels are above 180 mg/dL.
- * Thus, 1,5-AG evaluates hyperglycemic periods above 180 mg/dL for the previous two week period



GlycoMark

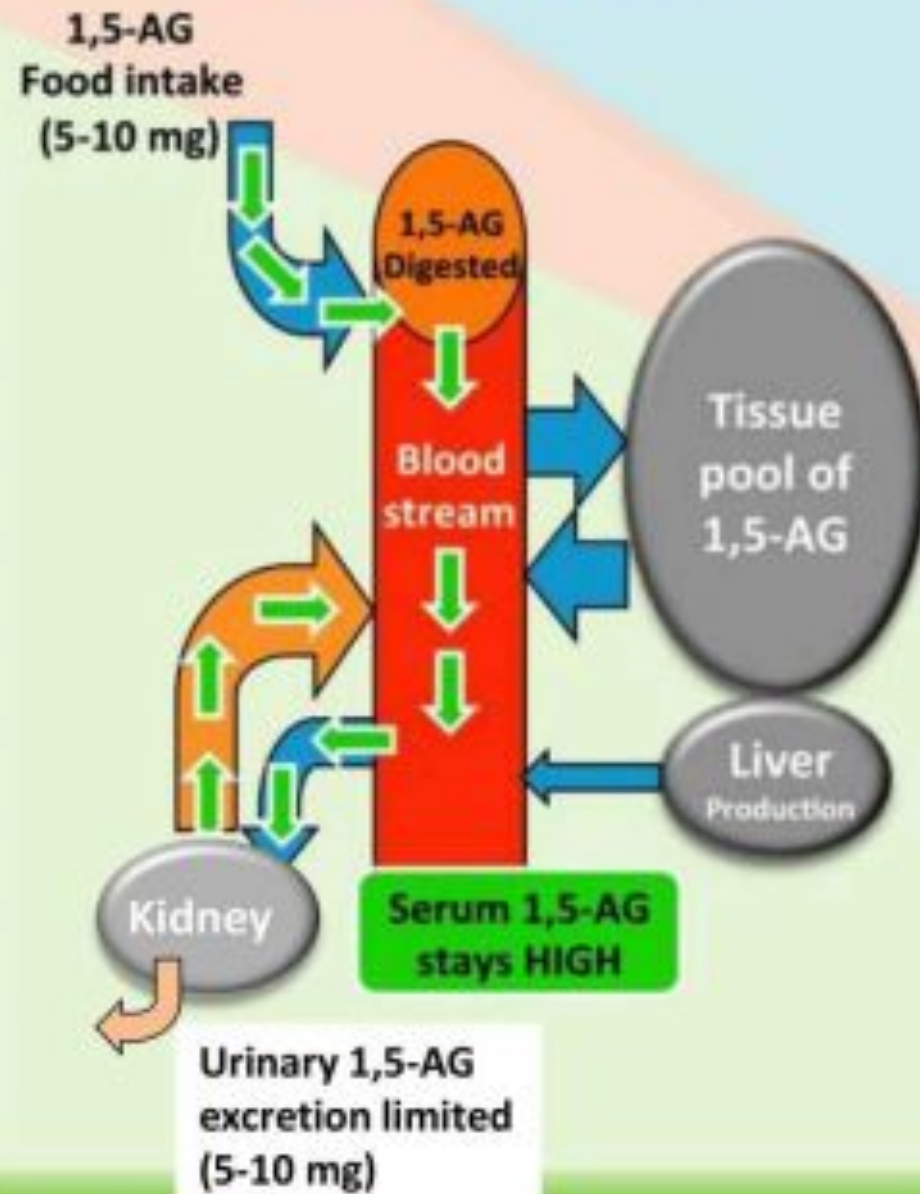
- * Compared to HbA1C
 - * A1C = quantity of glucose control
 - * 1,5 Anhydroglucitol = quality of glucose control



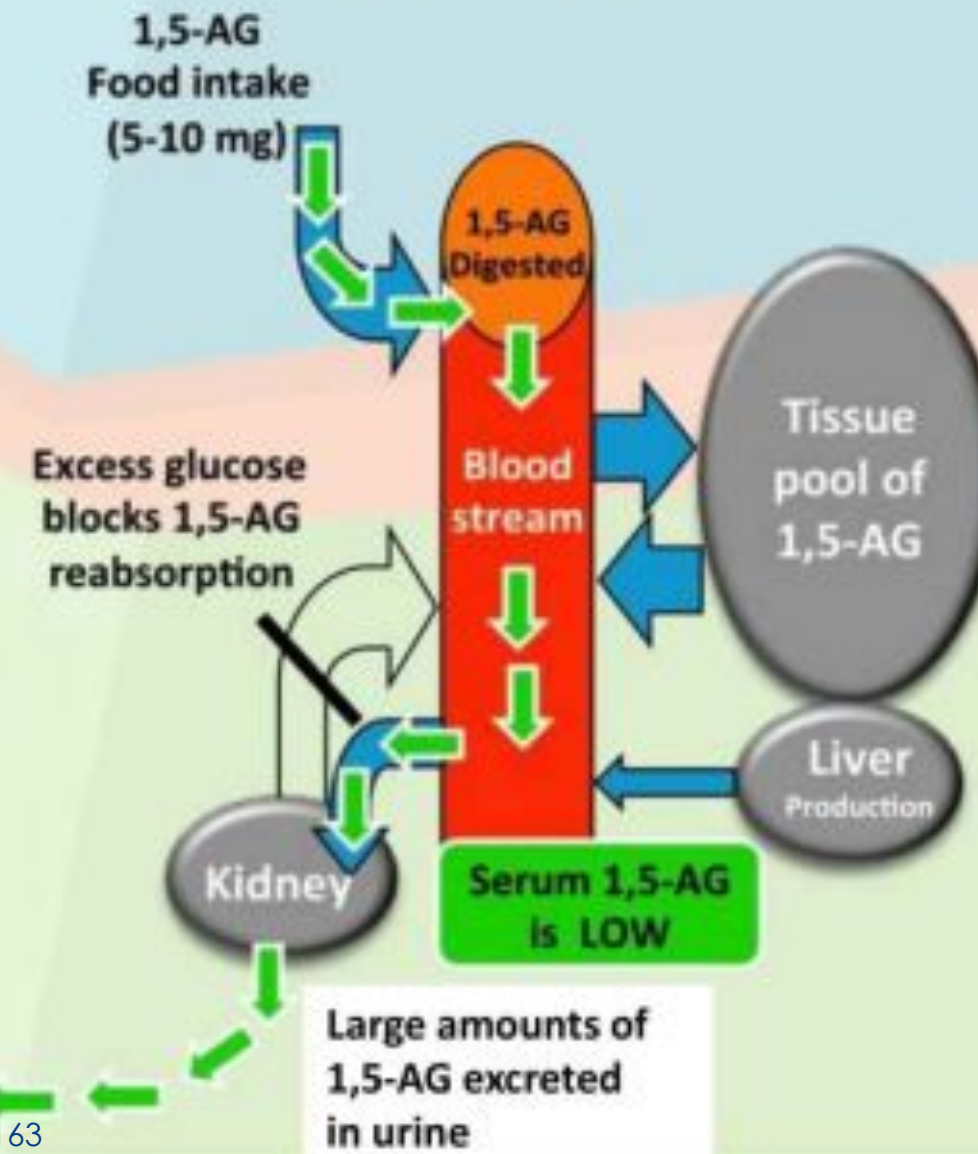
Physiology of 1,5-Anhydroglucitol (1,5-AG)

Why 1,5-AG decreases with hyperglycemia

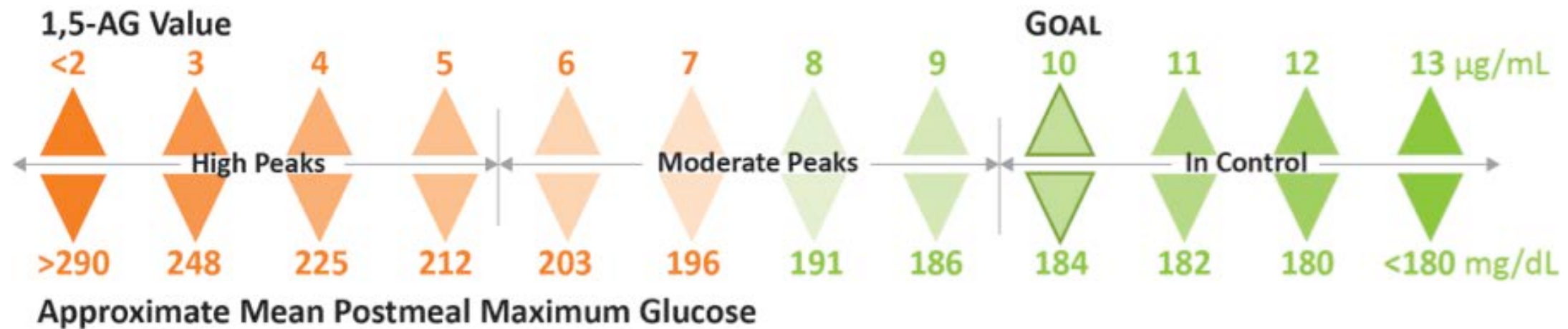
Normoglycemia



Hyperglycemia



GlycoMark ($\mu\text{g/mL}$)	Approximate Mean Postmeal Maximum Glucose (mg/dL)
> 12	< 180
10	185
8	190
6	200
4	225
< 2	> 290



Glycomark - LT

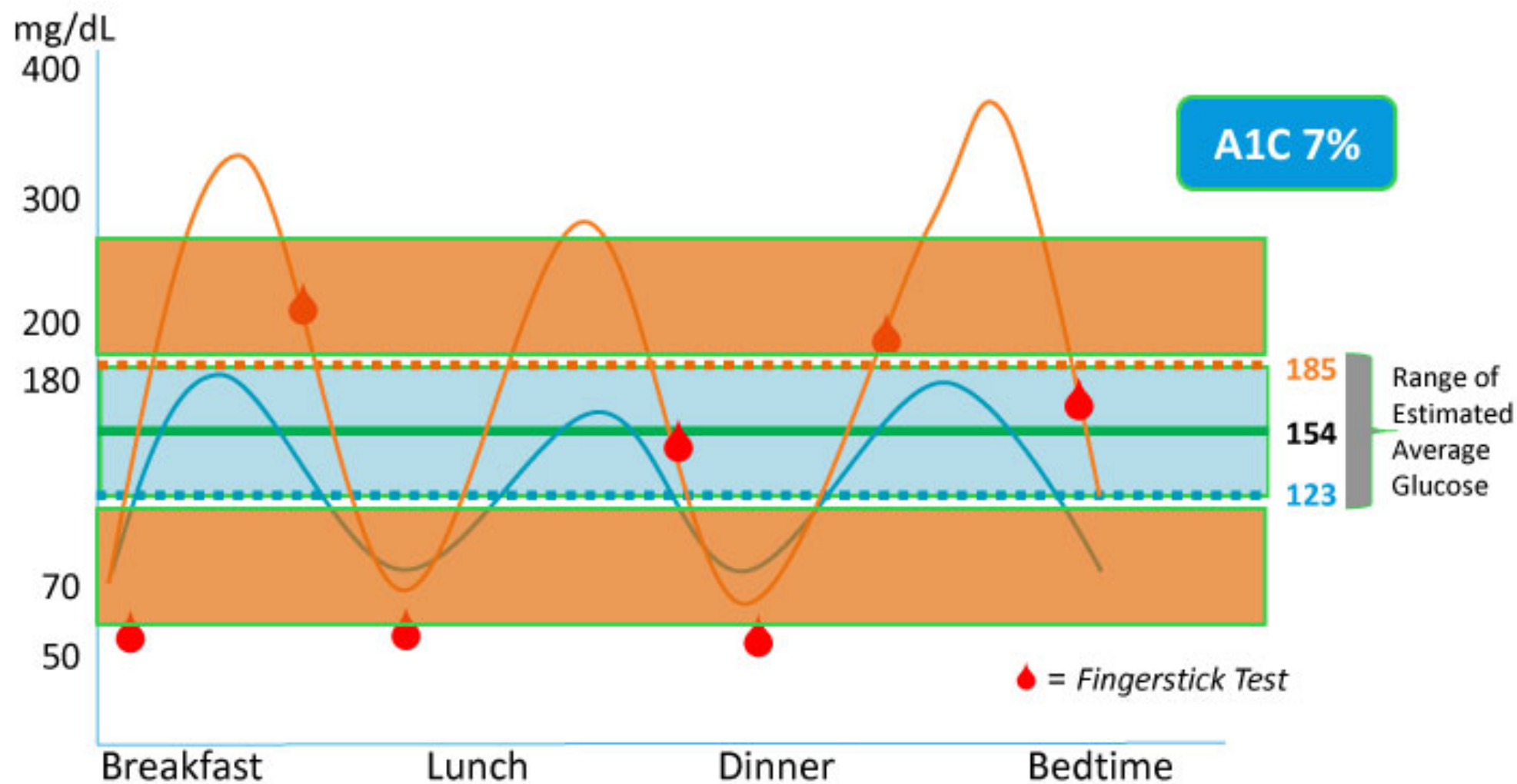
Glycomark	
Mean	15.3851145
Standard Error	0.259559569
Median	15
Mode	13.3
Standard Deviation	5.941590116
Minimum	1.1
Maximum	37.3
Sum	8061.8
Confidence Level(95.0%)	0.509907425

Out of 524 labs:

- 259 had a glycomark of 14.9 or below
 - 49% of members had a glycomark suggestive of elevated post-prandial glucose levels
- 35 had a glycomark of 7 or below
 - 7% of members have glucose above 200 after meals

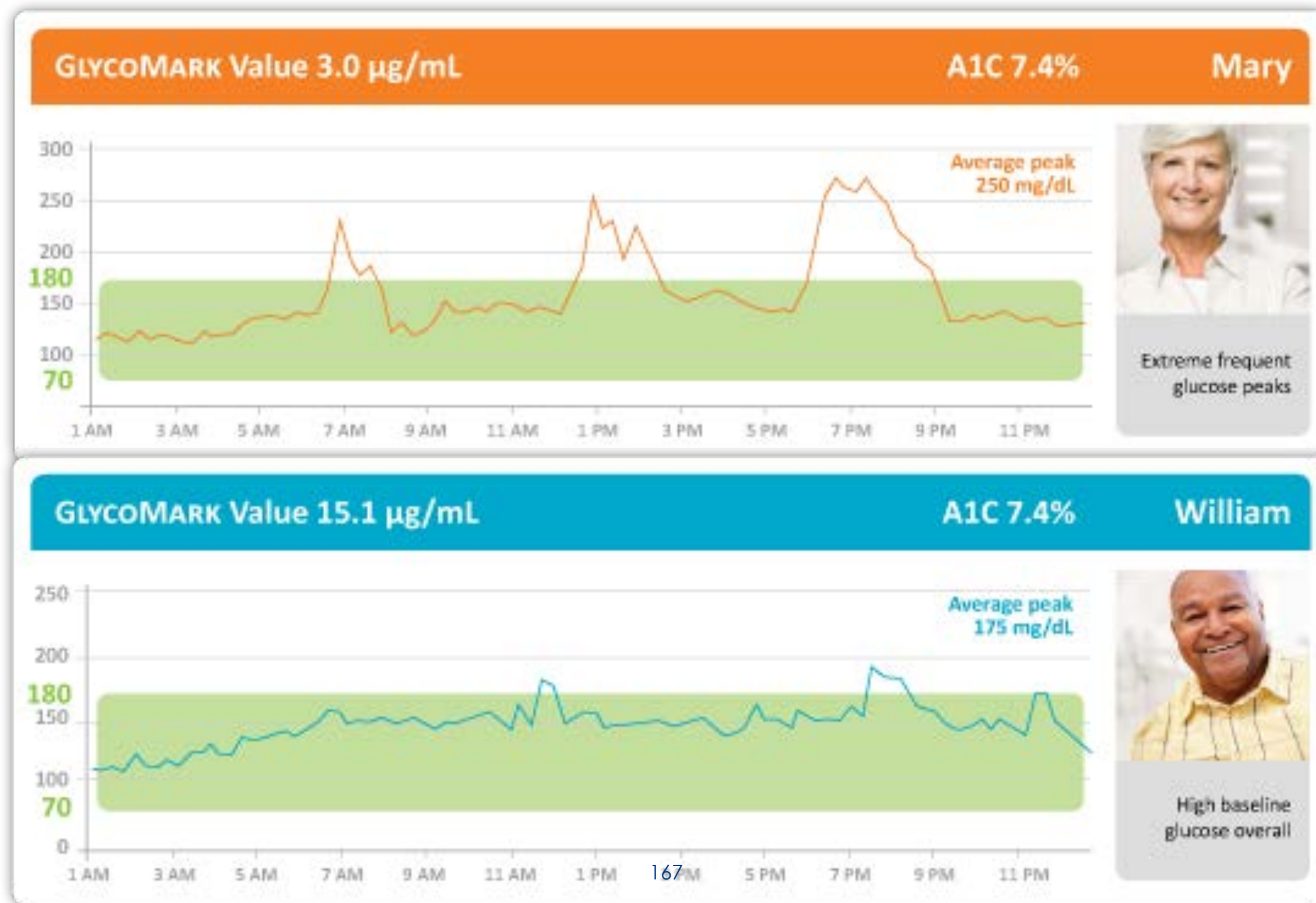
GlycoMark Tells You More

*A1C is a three-month average. Fingersticks are data points at the moment taken.
Only GlycoMark identifies maximum glucose spikes over the past two weeks.*

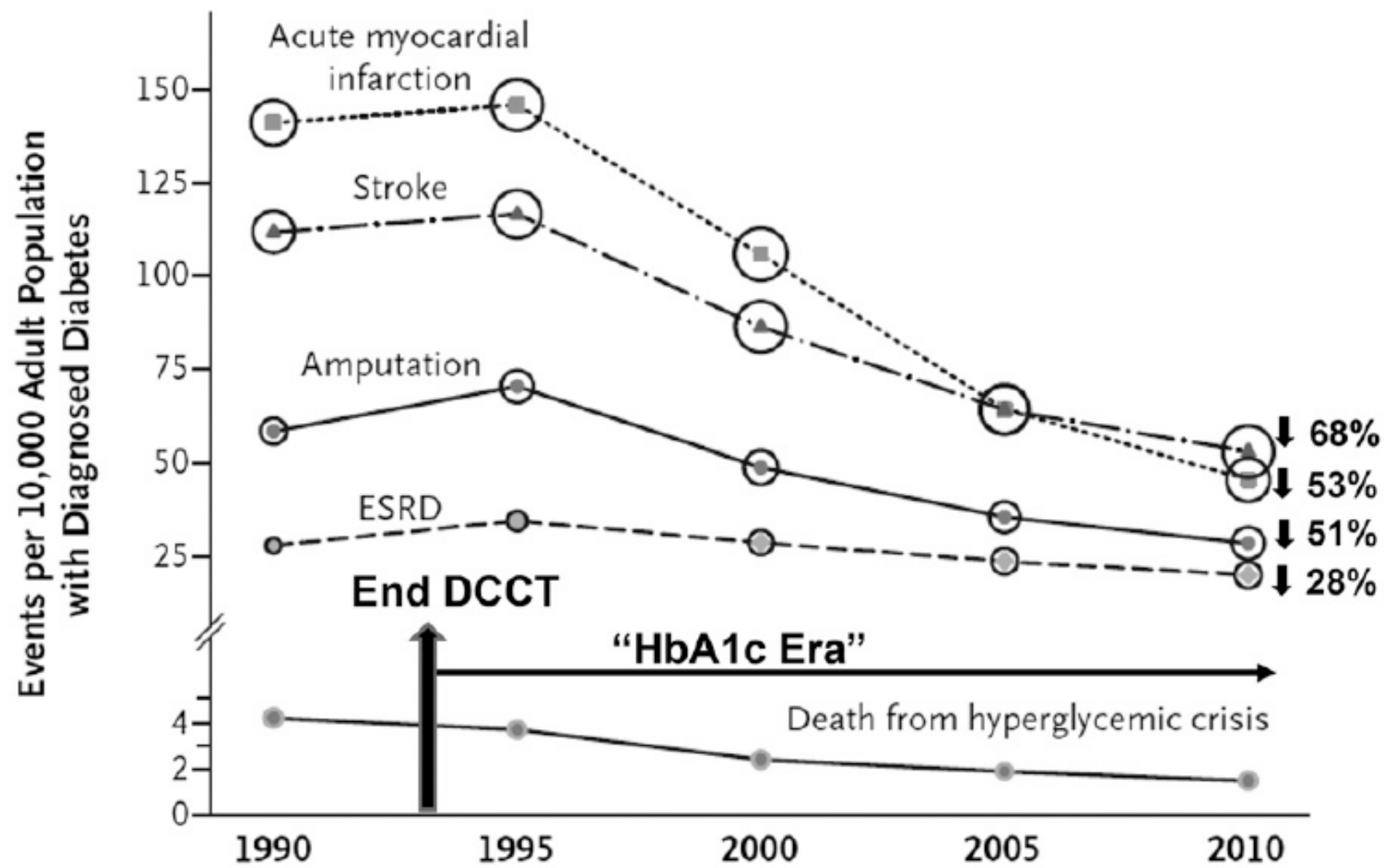


D Nathan et al, Translating the A1C Assay into Average Glucose Values,
Diabetes Care, Vol. 31, No. 8, August 2008

Glycemic Variability



Glycemic Variability – End of the A1C Era?



Glycemic Variability

- Increased oxidative stress
- Increases oxidative stress in adipocytes leading to less adiponectin and more resistin (highly pro-inflammatory)
- Hypoglycemic tendencies
 - Inflammation
- Decreased brown adipocyte thermogenesis
- **Association between obesity and glycemic variability**
- Micro- and macrovascular complications
- Mood, depression, and poor quality of life

Glucose Variability; Does It Matter?

Sarah E. Siegelar, Frits Holleman, Joost B. L. Hoekstra, and J. Hans DeVries

Academic Medical Centre, Department of Internal Medicine, 1105 AZ Amsterdam, The Netherlands

Overall lowering of glucose is of pivotal importance in the treatment of diabetes, with proven beneficial effects on microvascular and macrovascular outcomes. Still, patients with similar glycosylated hemoglobin levels and mean glucose values can have markedly different daily glucose excursions. The role of this glucose variability in pathophysiological pathways is the subject of debate. It is strongly related to oxidative stress *in vitro*,

Patients with similar mean glucose or glycosylated hemoglobin (HbA1c) values can have markedly different daily glucose profiles, with differences both in number and duration of glucose excursions.

In vitro, animal and human studies in experimental settings consistently report a deleterious effect of intermittent high glucose, either larger than or as large as constant high glucose, despite less total glucose exposure.

In comparison to the long-acting analog insulin glargine, the glucagon-like peptide-1 receptor agonist exenatide reduced glucose variability with a similar reduction in HbA1c.

Oscillating Glucose Is More Deleterious to Endothelial Function and Oxidative Stress Than Mean Glucose in Normal and Type 2 Diabetic Patients

Antonio Ceriello,¹ Katherine Esposito,² Ludovica Piconi,³ Michael A. Ihnat,⁴ Jessica E. Thorpe,⁴ Roberto Testa,⁵ Massimo Boemi,⁵ and Dario Giugliano²

OBJECTIVE—To explore the possibility that oscillating glucose may outweigh A1C levels in determining the risk for cardiovascular diabetes complications.

RESEARCH DESIGN AND METHODS—A euglycemic hyperglycemic clamp at 5, 10, and 15 mmol/l glucose was given in increasing steps as a single “spike” or oscillating between basal and high levels over 24 h in normal subjects and type 2 diabetic patients. Flow-mediated dilatation, a marker of endothelial function, and plasma 3-nitrotyrosine and 24-h urinary excretion rates of free 8-iso PGF₂α, two markers of oxidative stress, were measured over 48 h postclamp.

value alone. It is therefore unknown whether two individuals with the same mean blood glucose but extremes of glucose variability might have the same or different level of risk for complications.

In a 1995 report of the Diabetes Control and Complications Trial, the risk of retinopathy progression associated with a given level of mean A1C differed significantly between intensively and conventionally treated patients (4). It was suggested that this may be a consequence of larger glycemic excursions in the conventional group (4). However, with regard to cardiovascular complications, it has been reported that fasting plasma glucose instability is

In this study, we have been able to show that oscillating glucose, over a period of 24 h, is more damaging to endothelial function than stable constant high glucose. This is true not only when a subject is exposed to the same total amount of glucose for 24 h (i.e., 10 mmol/l clamp) but even when the total amount is higher (i.e., 15 mmol/l clamp). Finally, data suggest that oxidative stress plays a key role in all of these phenomena.

From the ¹Centre of Excellence in Diabetes and Endocrinology, University Hospital of Coventry and Warwickshire, Warwick Medical School, University of Warwick, Coventry, U.K.; the ²Division of Metabolic Diseases, Center of Excellence for Cardiovascular Diseases, 2nd University of Naples SUN, Naples, Italy; the ³Morpurgo-Hofman Research Laboratory on Aging, Udine, Italy; the ⁴Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma; and the ⁵Diabetology Unit, INRCA, Ancona, Italy.

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FMD, flow-mediated dilatation; PGF₂α, prostaglandin F₂α.

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normoglycemia. Below, the techniques used to attain these conditions are described.

To maintain euglycemia, endogenous insulin secretion was inhibited during all the experiments using somatostatin (Sandostatin; Novartis Pharma, Basel, Switzerland) (10). Somatostatin was infused in two phases: 1) as a bolus dose of 25 µg over 1 min given 5 min before the start of the experiment and 2) as a continuous maintenance dose of 1.0 µg/min (10). The hyperglycemic-euglycemic clamp methodology was a modification of the method used by Del Prato et al. (10).

Normalization of glycemia. Insulin and/or 5% glucose to keep blood glucose levels between 4 and 6 mmol/l were started (11). Blood glucose levels were determined every 5 min with adjustment of the intravenous insulin infusion until steady-state glucose levels were between 4 and 6 mmol/l. At the steady state, venous glucose samples were drawn every 30 min.

Control study with somatostatin. Five normal subjects and five diabetic patients underwent a control study with 3 h somatostatin infusion alone, and endothelial function and nitrotyrosine plasma levels were evaluated. No change in these parameters was found.

Protocols. Two different clamp protocols were planned, and the diabetic patients and the normal subjects participating in each protocol were matched for age, sex, and BMI and for fasting glycemia and A1C in the case of diabetes.

Oscillatory Function Normal

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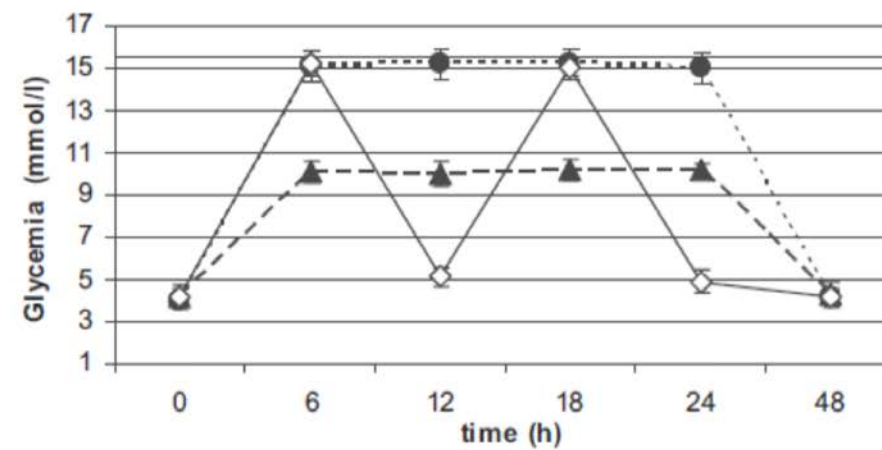
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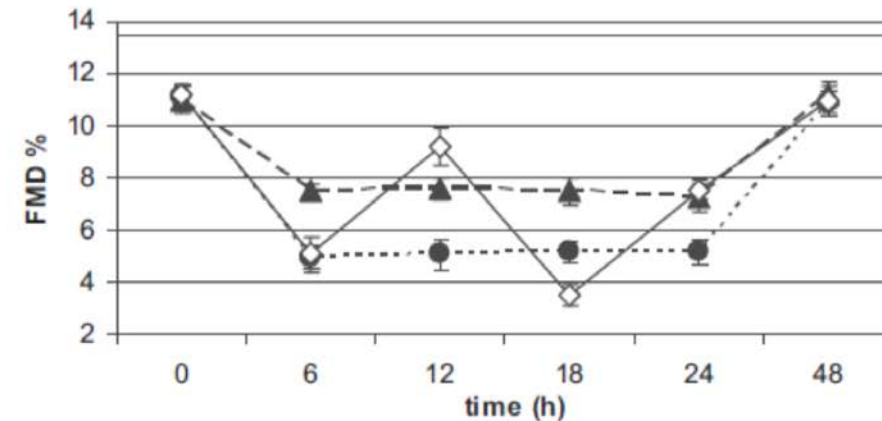
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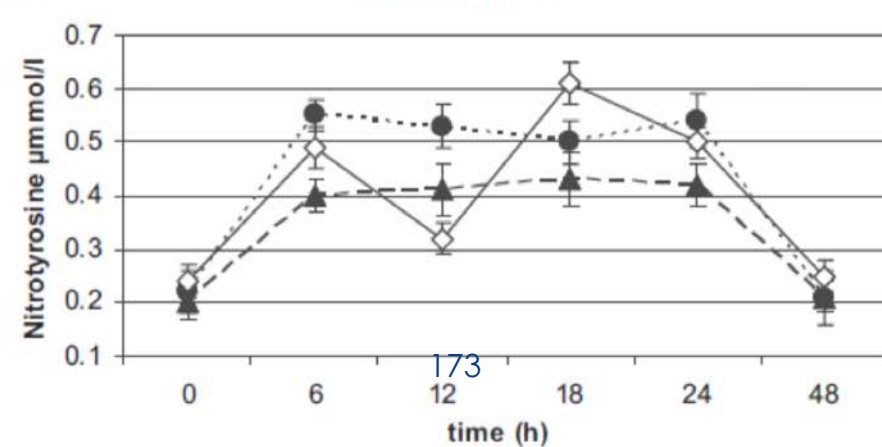
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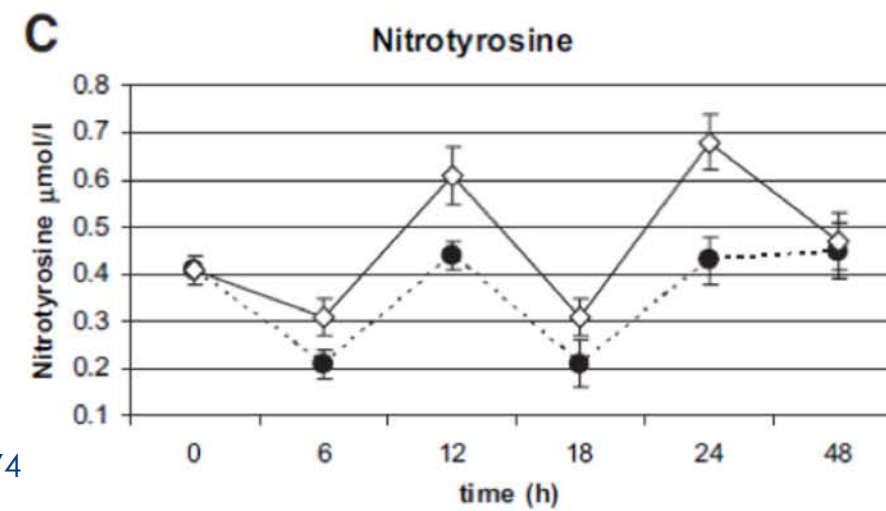
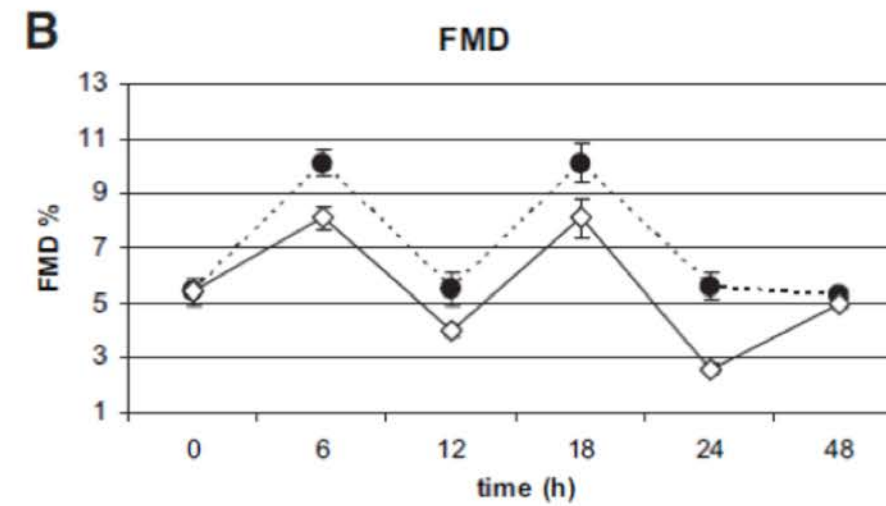
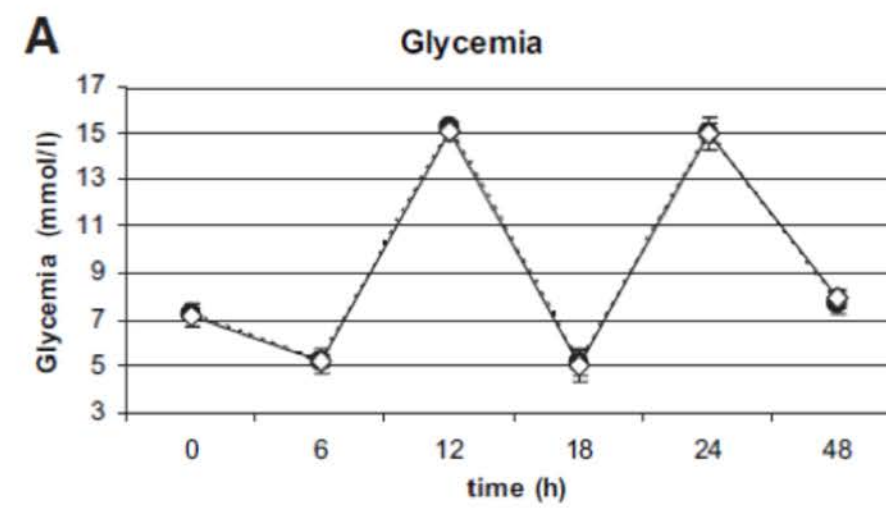
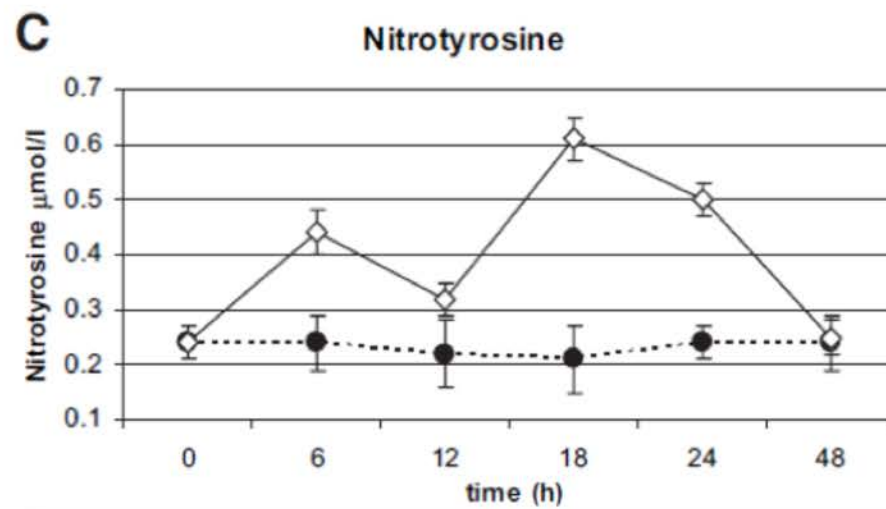
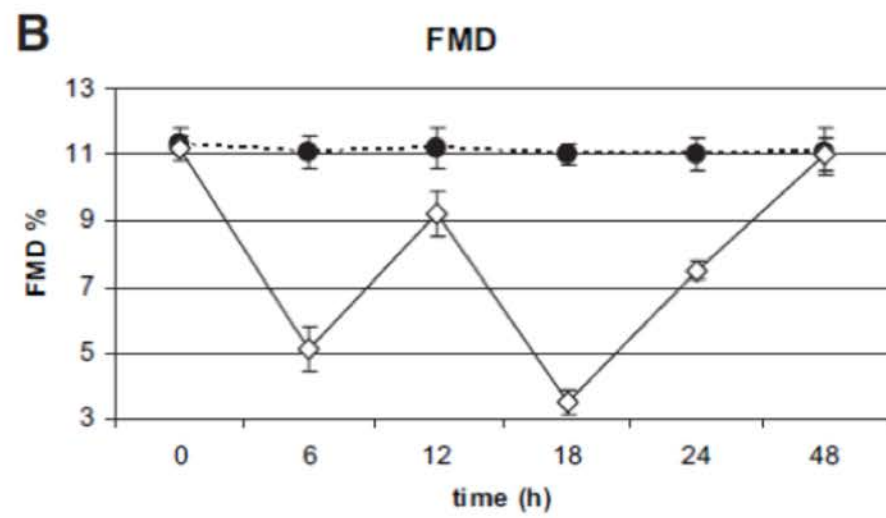
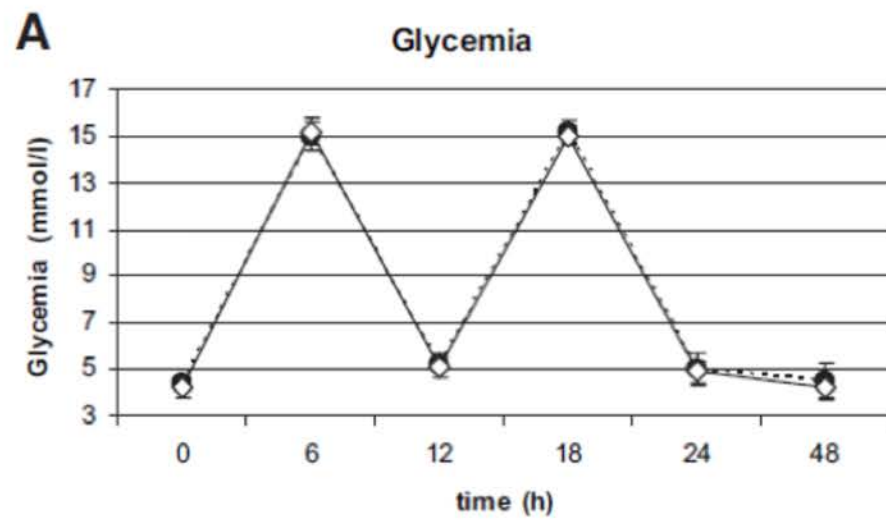
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Glycemic Variability

Should we and can we prevent it?

The role of glucose variability from peaks to nadirs is less documented, but there are many reasons to think that both upward (postprandial) and downward (interprandial) acute fluctuations of glucose around a mean value activate the oxidative stress.

As a consequence, it is strongly suggested that a global antidiabetic strategy should be aimed at reducing to a minimum the different components of dysglycemia (i.e., A1C, fasting and postprandial glucose, as well as glucose variability).

All the therapeutic agents that act on postprandial glucose excursions seem of particular interest for reducing the latter parameter (i.e., the glucose instability).

Particular attention should be paid to such emerging therapeutic agents as the glucagon-like peptide 1 agonists and the dipeptidyl peptidase (DPP)-IV inhibitors that act through the incretin pathway.

Intermittent high glucose exacerbates the aberrant production of adiponectin and resistin through mitochondrial superoxide overproduction in adipocytes

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Abstract

Hypoadiponectinemia and hyperresistinemia may be important in mediating signals from adipocytes to insulin-sensitive tissue and vasculature. However, the mechanism that mediates the aberrant production of adipokines remains poorly understood. In this study, we have investigated the effect of intermittent high glucose on the expression of adiponectin and resistin, and the production of 8-hydroxydeoxyguanosine (8-OHdG) and nitrotyrosine in the adipocytes, either in the presence or in the absence of Mn(II) tetrakis(4-benzoic acid) porphyrin chloride (MnTBAP) or thenoyltrifluoroacetone (TTFA). 3T3-L1 adipocytes were incubated for 72 h in media containing different glucose concentrations: 5 mmol/l, 20 mmol/l, 5 mmol/l alternating with 20 mmol/l glucose, with or without MnTBAP and TTFA. We measured the expression of resistin and adiponectin. The production of nitrotyrosine and 8-OHdG as oxidative stress parameter was measured. Both constant and intermittent high glucose significantly suppressed the expression and secretion of adiponectin, and increased expression and secretion of resistin in mature adipocytes compared to normal glucose conditions. However,

Intermittent high glucose exacerbates the aberrant production of adiponectin and resistin through reactive oxygen species overproduction at the mitochondrial transport chain level in adipocytes, indicating that glycemic variability has important pathological effects on the secretion of adipokines.

inhibitor-1, are linked to inflammation and the inflammatory response. The adipokines appear to be involved in a wide range of physiological processes, including hemostasis, lipid metabolism, blood pressure regulation, insulin sensitivity, and angiogenesis (Fruhbeck *et al.* 2001, Trayhurn & Wood 2004). The aberrant production of inflammatory adipokines is increasingly considered to be etiologically important in the development of diseases linked to obesity, particularly type 2 diabetes, cardiovascular diseases, and the metabolic syndrome (Hotamisligil 2003, Yudkin 2003). Thus, type 2 diabetes and atherosclerosis, as well as the other components of the metabolic syndrome, have been causally linked to inflammation.

Aberrant production of adipose-derived adipokines, especially hypoadiponectinemia and elevated resistin

increase expression of adipokines in adipocytes (Lin *et al.* 2005). Oxidative stress, endoplasmic reticulum stress, and nuclear factor kappa B (NF- κ B) activation pathways also operate in adipocytes (Van Gaal *et al.* 2006). Oxidative stress and inflammation in adipose tissue can be exacerbated by hyperglycemia (Lin *et al.* 2005).

The increased production of superoxide by the mitochondrial electron transport chain plays a causative role in hyperglycemic damage (Nishikawa *et al.* 2000). A possible involvement of this pathway in the hyperglycemia-induced aberrant production of inflammatory adipokines from adipocytes has not yet been reported. Therefore, the purpose of this study was to verify a possible connection between mitochondrial ROS production and aberrant production of adiponectin and resistin induced by both intermittent and

Glycemic Variability in Nondiabetic Morbidly Obese Persons: Results of an Observational Study and Review of the Literature

Sara J. Salkind, MS¹, Robert Huizenga, MD², Stephanie J. Fonda, PhD¹, M. Susan Walker, PhD¹, and Robert A. Vigersky, MD¹

Abstract

Glycemic variability (GV) is correlated with oxidative stress which may lead to increased cardiovascular risk and poor clinical outcomes in people with prediabetes and diabetes. We sought to understand whether morbidly obese persons without diabetes by standard criteria have dysglycemia as measured by GV. We performed an observational study of GV metrics and carotid intima media thickness (CIMT) in 21 morbidly obese normoglycemic and 15 morbidly obese prediabetic applicants to *The Biggest Loser* television show. The results were compared to previously published studies in normoglycemic nonobese and obese individuals. Glucose was measured with a masked continuous glucose monitor (CGM) over 3 to 8 days and

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SAGE

Normoglycemic and prediabetic morbidly obese individuals have higher GV compared with normal weight, nondiabetic individuals. We speculate that this may increase the risk for macrovascular disease through excessive oxidative stress.

It is hypothesized that glycemic variability (GV) may play a role in diabetic microvascular complications in people with type 2 diabetes through stimulation of oxidative stress (OS).^{1,2} In vitro data suggest that GV is more deleterious than consistently high glucose concentrations alone,^{3–5} but it is unclear what contribution GV makes to micro- and macrovascular complications in humans.⁶ If GV causes OS and is an independent risk factor for complications for people with type 2 diabetes, it is important to understand when GV starts to appear. Obesity is a major risk factor for type 2 diabetes and cardiovascular disease,⁷ which are both epidemic in the United States.^{8,9} People with morbid obesity provide a unique opportunity to study the relationship of obesity and GV.

Herein, we present data on CGM-derived GV in normoglycemic and prediabetic morbidly obese individuals. The GV of a pure population of morbidly obese individuals has not previously published. We then summarize these new findings about GV in the context of the extant literature on

CGM-derived GV in normal weight and obese people without diabetes.^{10–18}

Research Design and Methods

We performed a nonrandom, uncontrolled, observational study of 40 morbidly obese (BMI ≥ 40) applicants to the tenth season of *The Biggest Loser*, a television show where participants compete to lose weight over a 6- to 7-month period (<http://www.nbc.com/the-biggest-loser/>). This was a

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Serum 1,5-anhydro-D-glucitol levels predict first-ever cardiovascular disease: An 11-year population-based Cohort study in Japan, the Suita study

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ABSTRACT

Objective: Serum 1,5-anhydro-D-glucitol (1,5-AG) is well-known to be a useful clinical marker of both short-term glycemic status and postprandial hyperglycemia. In addition, previous epidemiological studies have shown that an increased postload glucose level in an oral glucose tolerance test is a risk factor for cardiovascular disease (CVD). However, no previous prospective study has reported the association

Our results suggest that measurement of serum 1,5-AG levels is useful to detect individuals, especially men, at higher risk for CVD, regardless of the presence or absence of diabetes.

The HR in the category with serum 1,5-AG levels of 14.0 µg/mL or less was 2.22 compared to the reference category (24.5 µg/mL or greater). Similar results were also shown with a sensitivity analysis in non-diabetic men. Conversely, no significant relationship between serum 1,5-AG levels and CVD risks was observed in women.

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Glycemic Variability Is an Independent Predictive Factor for Development of Hepatic Fibrosis in Nonalcoholic Fatty Liver Disease

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Abstract

Patients with nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) often have metabolic disorders including insulin resistance and type 2 diabetes mellitus (T2DM). We clarified the predictive factors in glucose metabolism for progression of hepatic fibrosis in patients with NAFLD by the 75-g oral glucose tolerance test (75gOGTT) and a continuous glucose monitoring system (CGMS). One hundred sixty-nine patients (68 female and 101 male patients) with biopsy-proven NAFLD with performance with 75gOGTT were enrolled and divided into four groups according to the stage of hepatic fibrosis (F0–3). The proportion of patients with T2DM significantly gradually increased, HbA1c and the homeostasis model assessment of insulin resistance were significantly elevated, and 1,5-anhydroglucitol (1,5-AG) was

Conclusion: Hyperinsulinemia and hyperglycemia, especially glycemic variability, are important predictive factors in glucose impairment for the progression of hepatic fibrosis in NAFLD.

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Introduction

Nonalcoholic fatty liver disease (NAFLD) includes a wide spectrum of liver diseases that range from simple steatosis, which is usually a benign and non-progressive condition, to nonalcoholic steatohepatitis (NASH), which can progress to liver cirrhosis (LC) and hepatocellular carcinoma in the absence of significant alcohol consumption [1–3]. The progression of hepatic fibrosis is an important predictive factor for the development of LC and hepatocellular carcinoma not only in patients with chronic hepatitis C, but also in those with NASH [4]. To inhibit the progression of hepatic fibrosis in NASH, it is important to clarify the predictive factors for progression of hepatic fibrosis.

NASH and NAFLD are considered to be hepatic manifestations of the metabolic syndrome including insulin resistance (IR) and abnormalities of glucose metabolism [5,6]. In accordance

with the increased prevalence of obesity and type 2 diabetes mellitus (T2DM) in the general population worldwide, the number of patients with NASH and NAFLD have also increased [7,8]. T2DM is considered to be an independent risk factor for the development of NASH and NAFLD [9,10], and hyperinsulinemia and hyperglycemia are common not only in obese patients, but also in non-obese, non-diabetic patients with NASH [11]. On the other hand, the presence of NASH and NAFLD themselves is also considered to be associated with a high risk of developing T2DM [12].

Postprandial hyperglycemia and glycemic variability were reported to involve progression of atherosclerosis through increase of oxidative stress, activation of inflammatory cytokines and inflammation [13–15]. Oxidative stress is well known as one of most important factor for inflammation and progression of hepatic fibrosis in NAFLD patients [16,17]. The continuous glucose



Glycemic Variability Is Associated With Reduced Cardiac Autonomic Modulation in Women With Type 2 Diabetes

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In patients with newly diagnosed and well-controlled type 2 diabetes, increased glycemic variability was associated with reduced cardiac autonomic modulation in women but not in men.

PATHOPHYSIOLOGY

$P < 0.001$, and RS ($P = 0.006$) were higher in men, whereas expiration–inspiration ($P < 0.001$), but not HF, was higher in women. In women, reduced cardiac autonomic modulation as assessed by the standard deviation of normal-to-normal intervals ($P = 0.001$), the root mean square of successive differences ($P = 0.018$), LF ($P < 0.001$), HF ($P = 0.005$), total power ($P = 0.008$), RS ratio ($P = 0.027$), and expiration-to-inspiration ratio ($P = 0.006$) was significantly associated with increased glycemic variability as assessed by MAGE. This was not the case in men. The association in women persisted in a multivariate regression analysis controlling for weight, mean heart rate, blood pressure (systolic), and triglycerides.

CONCLUSIONS

In patients with newly diagnosed and well-controlled type 2 diabetes, increased glycemic variability was associated with reduced cardiac autonomic modulation in women but not in men.

Evidence points toward a cardioprotective effect of sex hormones (estrogen and progesterone) in premenopausal women, one possibly mediated through improved cardiac autonomic balance both centrally and peripherally by suppression of sympathetic tone and elevation of parasympathetic tone (1,2). The presence of diabetes has been shown to reduce the sex difference and minimize cardioprotection in

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Clinical trial reg. no. NCT00674271, clinicaltrials.gov.

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Glycomark – the swiss army knife for blood glucose analysis...

Ability of 1,5-Anhydro-d-glucitol Values to Predict Coronary Artery Disease in a Non-Diabetic Population

Nobutaka IKEDA,¹ MD, Hisao HARA,¹ MD, and Yukio HIROI,¹ MD

SUMMARY

Increasing evidence has indicated that postprandial hyperglycemia affects coronary artery disease (CAD). The serum 1,5-anhydro-d-glucitol (1,5-AG) value is a useful clinical marker to evaluate short-term glycemic status and reflects glycemic excursions with greater sensitivity when compared with hemoglobinA1c (HbA1c), especially for patients in the postprandial state. The aim of this study was to evaluate the predictive value of 1,5-AG for CAD in patients without diabetes mellitus.

This study included 729 consecutive patients who had undergone their first coronary angiography. A total of 284 patients (246 diabetic patients and 38 patients with stage 4 or 5 chronic kidney disease) were excluded. The predictive values of 1,5-AG and HbA1c for CAD were evaluated by multivariable logistic regression analysis.

Patients with CAD demonstrated significantly lower 1,5-AG values and higher HbA1c values than did patients without CAD (18.6 $\mu\text{g/mL}$ [12.0, 23.3] versus 19.2 $\mu\text{g/mL}$ [14.4, 25.2], $P = 0.036$, and 5.7% [5.5, 5.9] versus 5.6% [5.4, 5.8], $P = 0.016$, respectively). In multivariable logistic regression analysis, the HbA1c values did not indicate a predictive value for the prevalence of CAD. In contrast, the 1,5-AG levels were still an independent predictor of CAD (adjusted odds ratio 0.96, 95% confidence interval 0.93-0.99, $P = 0.0097$).

Serum 1,5-AG is superior to HbA1c for predicting CAD prevalence in patients without diabetes mellitus. (Int

In multivariable logistic regression analysis, the HbA1c values did not indicate a predictive value for the prevalence of CAD. In contrast, the 1,5-AG levels were still an independent predictor of CAD (adjusted odds ratio 0.96, 95% confidence interval 0.93-0.99, $P = 0.0097$).

Serum 1,5-AG is superior to HbA1c for predicting CAD prevalence in patients without diabetes mellitus.

strated the association between postprandial hyperglycemia and mortality with no relation to fasting glucose levels.⁽¹⁾

The serum 1,5-anhydro-d-glucitol (1,5-AG) level rapidly decreases concomitantly with urinary glucose excretion and is

5 chronic kidney disease. Diabetes mellitus was defined as fasting plasma glucose ≥ 126 mg/dL and HbA1c $\geq 6.5\%$ National Glycohemoglobin Standardization Program (NGSP), or the use of an oral hypoglycemic agent or insulin. This study

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Ability of 1,5-Anhydro-d-glucitol Values to Predict Coronary Artery Disease in a Non-Diabetic Population

Nobutaka IKEDA,¹ MD, Hisao HARA,¹ MD, and Yukio HIROI,¹ MD

SUMMARY

Increasing evidence has indicated that postprandial hyperglycemia affects coronary artery disease (CAD). The serum 1,5-anhydro-d-glucitol (1,5-AG) value is a useful clinical marker to evaluate short-term glycemic status and reflects

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1,5-AG AND CORONARY DISEASE IN NON-DIABETIC ADULTS

589

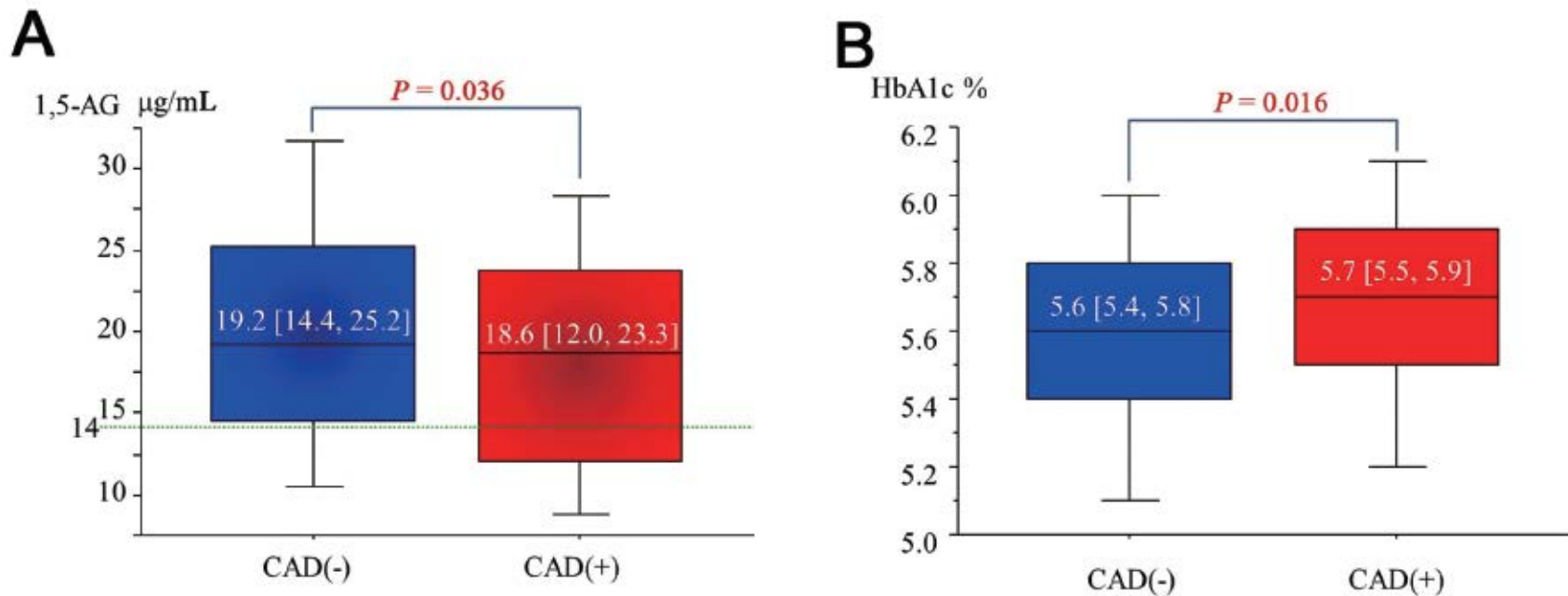


Figure. A: 1,5-AG values of patients with/without coronary artery disease (CAD). The widely accepted cut-off value for the detection of glycometabolic disorder (14 μg/mL) is within the 25th percentile of 1,5-AG values of the patients with and without CAD. B: HbA1c values of patients with/without CAD.

emia affects CVD progression.^{11,12} The DECODE trial demonstrated the association between postprandial hyperglycemia and mortality with no relation to fasting glucose levels.¹³ The serum 1,5-anhydro-d-glucitol (1,5-AG) level rapidly decreases concomitantly with urinary glucose excretion and is

excluding 246 diabetic patients and 38 patients with stage 4 or 5 chronic kidney disease. Diabetes mellitus was defined as fasting plasma glucose ≥ 126 mg/dL and HbA1c ≥ 6.5% National Glycohemoglobin Standardization Program (NGSP), or the use of an oral hypoglycemic agent or insulin. This study

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Original Article: Clinical Care and Delivery**Does serum 1,5-anhydroglucitol establish a relationship between improvements in HbA_{1c} and postprandial glucose excursions? Supportive evidence utilizing the differential effects between biphasic insulin aspart 30 and insulin glargine**

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Accepted 30 October 2007

Abstract**Aim** To investigate the relationship between changes in glycated haemoglobin (HbA_{1c}) and postprandial glucose

Even moderate elevations in HbA(1c) substantially lower 1,5-AG, suggesting that it can be most discriminating in identifying patients with excessive postprandial glucose excursions at HbA(1c) levels that approach the upper end of the normal range.

1,5-AG, suggesting that it can be most discriminating in identifying patients with excessive postprandial glucose excursions at HbA_{1c} levels that approach the upper end of the normal range.

Diabet. Med. 25, 200–205 (2008)

Keywords basal, glycaemic control, insulin analogues, target, Type 2 diabetes

Abbreviations 1,5-AG, 1,5-anhydroglucitol; BAsp 30, biphasic insulin aspart 30; FPG, fasting plasma glucose; HbA_{1c}, glycated haemoglobin; IGlar, insulin glargine; INITIATE, INITiation of Insulin to reach HbA_{1c} Target; PPG, postprandial glucose; PPG_{ave}, average change in postprandial plasma glucose

Introduction

Glycated haemoglobin (HbA_{1c}), does not discriminate between the relative contributions of fasting and postprandial glucose to mean glycaemia. Moreover, as glycation of haemoglobin takes several months to achieve steady state, HbA_{1c} provides

relatively little information about glycaemic control over shorter periods. Plasma 1,5-anhydroglucitol (1,5-AG), a major 6-carbon unmetabolizable dietary monosaccharide structurally resembling glucose, is maintained at a constant level during normoglycaemia; it falls once the glucosuric threshold is exceeded (usually 10 mmol/l) because of competitive inhibition of renal tubular absorption by glucose [1–4]. This analyte has been proposed as a marker for postprandial hyperglycaemia [2] and may provide a more complete view of an individual's glycaemic status than either random discrete glucose measurements or

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1,5-anhydroglucitol (GlycoMark™) as a marker of short-term glycemic control and glycemic excursions

Table 1. Comparison of glycemic markers.

Parameter	Hemoglobin A1C	Fructosamine	1,5-anhydroglucitol
Time required for significant change	1–3 months	1–2 weeks	1–3 days
Reflection of mean glucose	++	++	+
Reflection of glucose excursions/postprandial glucose	+	+	++
Association with complications	++	NA	NA
Variance	Small	Small	Large
Greatest degree of change is found during	Moderate-to-severe hyperglycemia	Moderate-to-severe hyperglycemia	Mild-to-moderate hyperglycemia

fewer than 30% were able to maintain that target over time [4]. In routine clinical practice, glycemic control may be even more difficult to achieve, and despite recent advances in therapy, only 50% of patients with diabetes reach recommended A1C targets [5]. Further complicating the matter are the more recent calls that advocate for normoglycemia (A1C <6.0%) where it can be done safely [3]. Quality improvement initiatives may benefit patients with the worst glycemic control [6], but patients with more modest control (A1C >8%) pose a tremendous challenge to clinicians [7].

Glycemic monitoring is of paramount importance for adjusting various treatment modalities. Conventional methods of assessing glycemic

mortality than fasting glucose measurements [9,10]. Therefore, quantification of glycemic fluctuations (e.g., glycemic variability, PPG or glucose excursions) might predict macrovascular disease better than mean glucose. *In vitro* data suggest that intermittent glycemic excursions may be more harmful to endothelial cells than chronic hyperglycemia, as the latter may induce relative conditioning of cellular inflammatory responses [11–13]. Oxidative stress, an important mediator of hyperglycemia-mediated cellular damage, is also associated with glycemic variability in patients with diabetes, independent of mean glucose [14]. A prospective randomized controlled study demonstrated that repaglinide,

Circulating 1,5-Anhydroglucitol Levels in Adult Patients With Diabetes Reflect Longitudinal Changes of Glycemia

A U.S. trial of the GlycoMark assay

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WILLIAM NOWATZKE, PHD¹
SHANNON HOUGHTON, MD¹

ERIKA B. AMMIRATI, BS²
THERESA GAUTILLE, RN²
MARK J. SARNO, BA²

OBJECTIVE — 1,5-Anhydroglucitol (1,5AG) is a major circulating polyol arising primarily from ingestion and excreted competitively with glucose. Japanese studies have demonstrated reduced concentrations of 1,5AG in serum in hyperglycemic patients in comparison with euglycemic subjects and a gradual normalization of 1,5AG values for patients responding to antihyperglycemic therapies. In this first U.S. study, we assessed the ability of 1,5AG measurements to monitor glycemic control in a cohort of 77 patients with diabetes (22 with type 1 diabetes, 55 with type 2 diabetes) who presented with suboptimal glycemic control at baseline

glycemia is of paramount importance because good metabolic control can reduce the incidence of micro- and macrovascular complications (2–5).

In recent years, various biochemical markers have been cleared by the U.S. Food and Drug Administration (FDA) for assessment of glycemia. These include self-monitored blood glucose methods and assays for HbA_{1c} and fructosamine. These tests differ in the time period in which measured values reflect glycemia.

We conclude that 1,5AG responds sensitively and rapidly to changes in glycemia and monitors glycemic control in accordance with established markers. (More rapidly than HbA1C.)

89.6% of patients displayed longitudinal changes of 1,5AG from baseline to week 8 in concordance with HbA_{1c}. 1,5AG was also highly correlated with HbA_{1c} and fructosamine (Spearman $\rho = -0.6459$ and -0.6751 , respectively; both $P < 0.0001$).

CONCLUSIONS — We conclude that 1,5AG responds sensitively and rapidly to changes in glycemia and monitors glycemic control in accordance with established markers.

Diabetes Care 27:1859–1865, 2004

The incidence of diabetes is clearly a serious health concern. During the period 1991–2000, the incidence in the U.S. increased by 49% (4.9–7.3% of the total population), and this was correlated with a 61% increase in obesity during the same period (1,2). For those afflicted with diabetes, control of their

ever, despite the demonstrated use of HbA_{1c} measurements, the slow rate of change may contribute to delays in modification of therapy. Thus, a marker that responds rapidly and significantly to changes in glycemia, that is metabolically stable, that demonstrates low biological variability, and that can be easily measured would be useful in management of patients with diabetes.

1,5-Anhydroglucitol (1,5AG) has been recently proposed as a marker conforming to these criteria (7). 1,5AG was first discovered in the plant family *Polygala senega* in 1888. The structure was identified in 1943, and the presence of the compound in human blood (8) and cerebrospinal fluid (9) was established in 1972 and 1973, respectively. Research studies have shown that 1,5AG originates mostly from foods with a mean intake of ~4.4 mg/day, that the closed pyran ring structure confers metabolic stability, that the rate of intake is matched by the daily excretion rate, and that a bodily pool of ~500–1,000 mg of 1,5AG is constantly

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T.G.C. and W.N. are currently affiliated with Linco Research, St. Louis, Missouri.

Abbreviations: 1,5AG, 1,5-anhydroglucitol; FDA, Food and Drug Administration; PROD, pyranose oxidase.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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ORIGINAL ARTICLE

1,5-Anhydroglucitol Is Associated with Early-Phase Insulin Secretion in Chinese Patients with Newly Diagnosed Type 2 Diabetes Mellitus

Xiaojing Ma, MD,* Yaping Hao, MD,* Xiang Hu, MD, Yuqi Luo, MD, Zixuan Deng, MD, Jian Zhou, MD, PhD, Yuqian Bao, MD, and Weiping Jia, MD, PhD

Abstract

Background: The goal of the present study was to explore the correlations of 1,5-anhydroglucitol (1,5-AG), glycated hemoglobin (HbA1c), and glycated albumin (GA) with insulin sensitivity and secretion.

Increased 1,5-AG quartiles were accompanied by trends toward a decreased HOMA-IR and an increased HOMA- β and IGI. 1,5-AG was negatively associated with HOMA-IR and positively associated with HOMA- β and IGI. 1,5-AG was independently related to HOMA-IR and HOMA- β and exhibited an independent positive association with IGI. Additionally, both HbA1c and GA were independently correlated with HOMA-IR and HOMA- β .

1,5-AG is not only correlated with basal insulin sensitivity and secretion, but also closely associated with early-phase insulin secretion in Chinese patients with newly diagnosed type 2 diabetes mellitus.

Glycomark

Hyperglycemia and glycemic variability have been linked to diabetes-related health complications including:

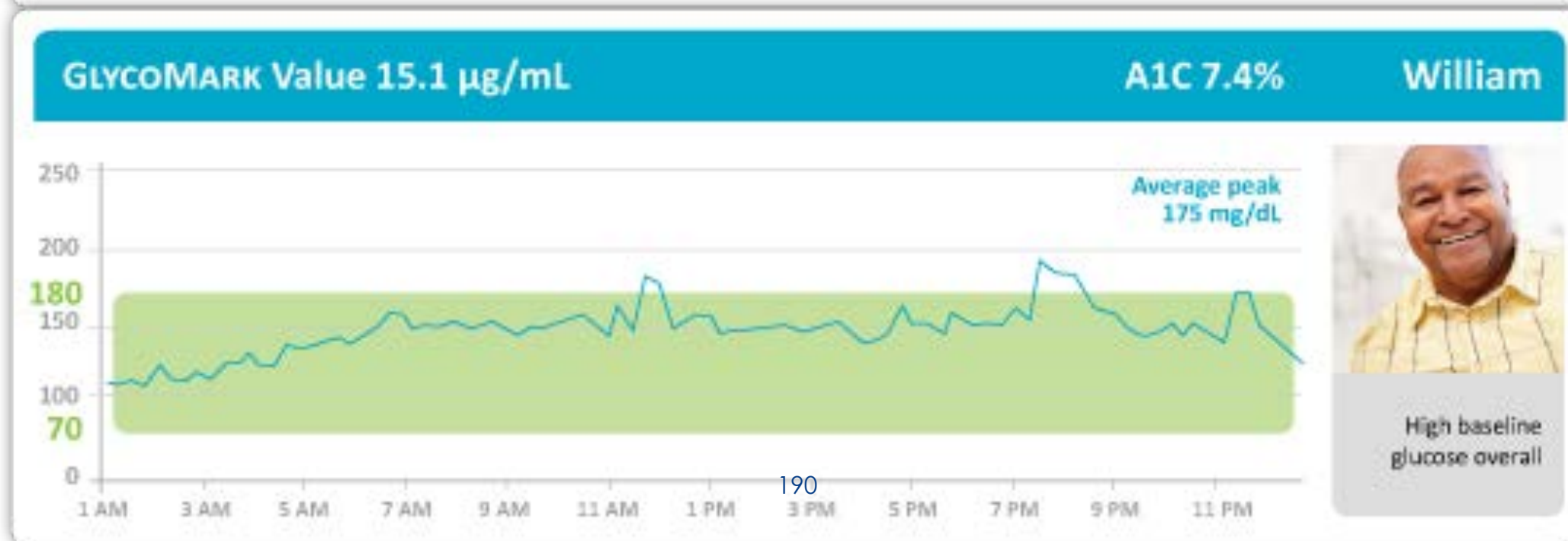
- * vascular damage (reduced flow-mediated dilation and coronary lumen diameter; increased carotid artery stiffness and carotid intima-media thickness)
- * oxidative stress (plasma 3-nitrotyrosine and 24-h urinary excretion rates of free 8-iso PGF₂)
- * increased inflammatory markers (C-Reactive Protein, Interleukin 6)
- * poor cardiovascular outcomes (repeat MI, acute heart failure)
- * stroke
- * dementia
- * increased risk of death from cardiovascular causes

Glycomark

1,5-anhydroglucitol in the blood and yielding a precise one- to two-week profile of average daily maximum blood glucose, thus:

- * identify patients with A1Cs at 8% or less who have had more frequent and extreme hyperglycemic excursions in the past one to two weeks
- * recognize recent glycemic deterioration before changes are visible with A1C
- * record improvements in therapy changes within two to four weeks
- * measure and positively reinforce adherence to dietary and lifestyle changes

Glycomark/First Phase Insulin Response



Homeostatic Model Assessment (HOMA)

- * HOMA2 estimates steady state beta cell function (%B) and insulin sensitivity (%S), as percentages of a normal reference population. These measures correspond well, but are not necessarily equivalent, to non-steady state estimates of beta cell function and insulin sensitivity derived from stimulatory models such as the hyperinsulinaemic clamp, the hyperglycaemic clamp, the intravenous glucose tolerance test, and the oral glucose tolerance test.

Homeostatic Model Assessment (HOMA)

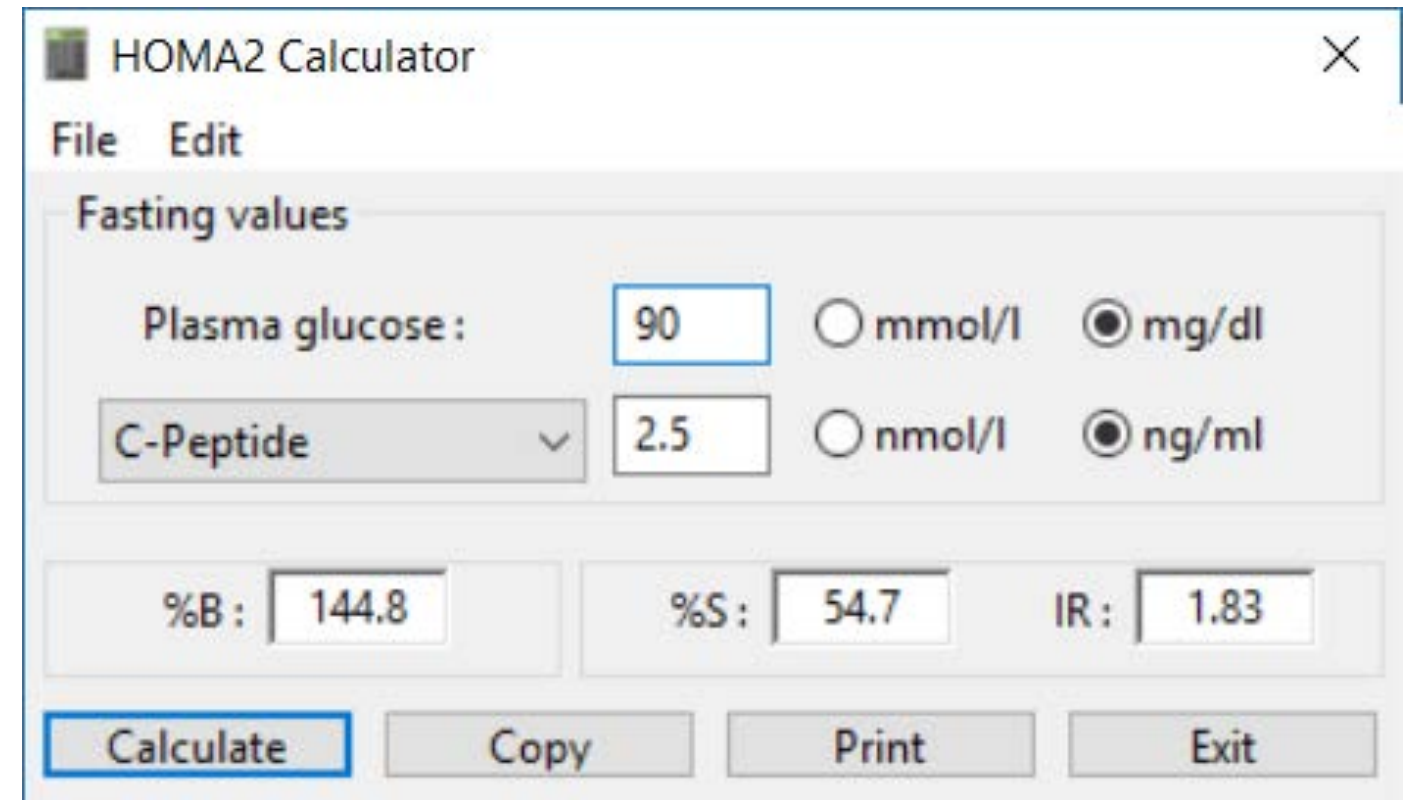
- * Traditionally used serum insulin, but has been validated to use C-peptide as well
 - * $\text{Homa-IR (CP)} = 1.5 + \text{fasting blood glucose} \times \text{fasting C-peptide} / 2800$
 - * $\text{Homa-islet (CP-Normal)} = 0.27 \times \text{fasting C-peptide} / (\text{fasting blood glucose} - 3.5) + 50$
- * <https://www.dtu.ox.ac.uk/homacalculator/>

Homeostatic Model Assessment (HOMA)

Insulin resistance = <1.8

Beta cell (%B) = <145

Insulin Sensitivity (%S) = > 55



The screenshot shows the HOMA2 Calculator window. It has a menu bar with 'File' and 'Edit'. Under 'Fasting values', there are two rows of input fields. The first row is 'Plasma glucose' with a value of 90, and units ☐ mmol/l and ☒ mg/dl. The second row is 'C-Peptide' with a value of 2.5, and units ☐ nmol/l and ☒ ng/ml. Below these, the calculated results are displayed: %B: 144.8, %S: 54.7, and IR: 1.83. At the bottom, there are four buttons: 'Calculate', 'Copy', 'Print', and 'Exit'.

Input	Value	Unit
Plasma glucose	90	mg/dl
C-Peptide	2.5	ng/ml

Output	Value
%B	144.8
%S	54.7
IR	1.83

Homeostatic Model Assessment (HOMA)

HOMA2 Calculator

File Edit

Fasting values

Plasma glucose : 90 ☐ mmol/l ☒ mg/dl

C-Peptide 2.5 ☐ nmol/l ☒ ng/ml

%B : 144.8 %S : 54.7 IR : 1.83

Calculate Copy Print Exit

HOMA2 Calculator

File Edit

Fasting values

Plasma glucose : 90 ☐ mmol/l ☒ mg/dl

C-Peptide 1.0 ☐ nmol/l ☒ ng/ml

%B : 77.1 %S : 136.4 IR : 0.73

Calculate Copy Print Exit

HOMA2 Calculator

File Edit

Fasting values

Plasma glucose : 90 ☐ mmol/l ☒ mg/dl

C-Peptide 4.0 ☐ nmol/l ☒ ng/ml

%B : 201.4 %S : 34.2 IR : 2.92

Calculate Copy Print Exit

STEP 1 - Glycomark

If Glycomark is $<15 \mu\text{g/mL}$:

1. Focus on diet – quality of food (macronutrient ratio) and quantity of food
2. GLP-1 stimulators (pre-meal)
 1. Fish oil
 2. Pea protein, glutamine
 3. Quercetin
 4. Bile acid support
 5. MUFA
 6. Chew food thoroughly
 7. Olive leaf extract



Glucose Variability in a 26-Week Randomized Comparison of Mealtime Treatment With Rapid-Acting Insulin Versus GLP-1 Agonist in Participants With Type 2 Diabetes at High Cardiovascular Risk

*The FLAT-SUGAR Trial Investigators**

DOI: 10.2337/dc15-2782

OBJECTIVE

A1C is associated with diabetes complications but does not reflect glycemic variability (GV), which may worsen outcomes by inducing inflammation, oxidative stress, and cardiac arrhythmias. We tested whether a glucagon-like peptide 1 agonist-based regimen can reduce GV and cardiometabolic risk markers while maintaining similar A1C levels in people with insulin-requiring type 2 diabetes and high cardiovascular risk.

RESEARCH DESIGN AND METHODS

After run-in on metformin and basal-bolus insulin (BBI), 102 participants continued metformin and basal insulin and were randomized to exenatide dosing before the two largest meals glucagon-like peptide-1 receptor agonist and insulin (GUPUII group) or continuation of rapid-acting insulin analogs (BBI group). Indices of GV by continuous glucose monitoring (CGM), hypoglycemia, weight, risk markers, and cardiac arrhythmias were assessed. The primary end point was change in glucose coefficients of variation (CV) by CGM from baseline to 26 weeks.

RESULTS

At randomization, the median A1C was 7.3% (57 mmol/mol) for GLIPULIN and 7.4% (56.3 mmol/mol) for BBI, and glucose CVs were 30.3 for BBI and 31.9 for GLIPULIN. At 26 weeks, A1C levels were similar (7.1% [54 mmol/mol] vs. 7.2% [55 mmol/mol]), whereas mean CV improved with GLIPULIN (−2.4 vs. 0.4, $P = 0.047$). Other GV indices followed similar nonsignificant patterns of improvement with GLIPULIN. There were no differences in hypoglycemic events during CGM or arrhythmias during electrocardiographic monitoring. On-trial changes in body weight (−4.8 kg vs. +0.7 kg, $P < 0.003$), alanine aminotransferase ($P = 0.0002$), and serum amyloid A ($P = 0.023$) favored GLIPULIN.

CONCLUSIONS

GLIPULIN reduced GV, weight, and some cardiometabolic risk markers while maintaining equivalent A1C levels versus BBI and might improve clinical outcomes in a larger trial.

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*The members of the Writing Committee of the FLAT-SUGAR Trial are listed in the appendix.

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STEP 2 – Hemoglobin A1C

If HbA1C is above 5.3%:

1. Exercise – skeletal muscle contraction + steady state cardio
2. Diet – macronutrient ratio, quantity of food
 - * Paleo-Mediterranean
3. Supplementation - Same supplements as Glycomark protocol
 - * Berberine
 - * Gymnema
 - * Banaba leaf
 - * Bitter melon
 - * Alpha-lipoic acid
 - * Chromium
 - * Vanadium

STEP 2 – Hemoglobin A1C

If HbA1C is above 5.7%:

1. Exercise – skeletal muscle contraction + steady state cardio
2. Diet – macronutrient ratio, quantity of food
 - * Paleo-Mediterranean
3. Supplementation - Same supplements as Glycomark protocol
 - * Lipotropic factors – choline, methionine, TMG

STEP 3 – C-Peptide

If C-peptide is above 2.5:

1. Mitochondrial support

- * Glutathione
- * NAC
- * ALA
- * Carnitine

If C-peptide is below 1.1 + elevated A1C:

1. GABA

Suppress Glucagon

- * Gamma amino butyric acid (GABA)
 - * With meals

Intra-islet insulin suppresses glucagon release via GABA-GABA_A receptor system

Elaine Xu,^{1,2,7} Mohan Kumar,^{1,2,7} Yi Zhang,^{1,2} William Ju,^{1,2} Toshiyuki Obata,³ Nina Zhang,² Shiyong Liu,² Anna Wendt,⁴ Shaoping Deng,⁵ Yousuke Ebina,³ Michael B. Wheeler,¹ Matthias Braun,^{4,6} and Qinghua Wang^{1,2,*}

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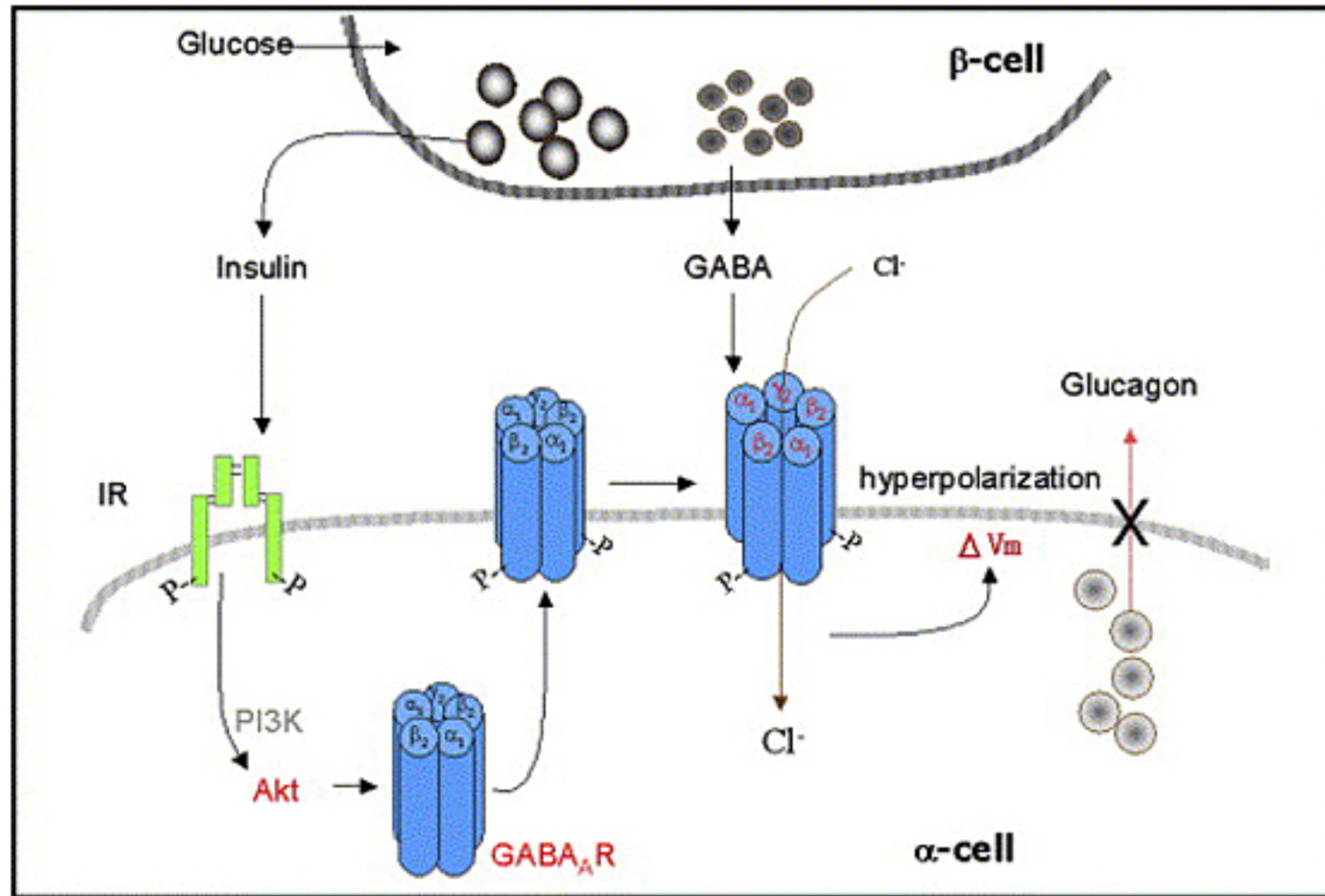
The intra-islet action of insulin is essential to exert the effect of glucose on the alpha cells since, in the absence of insulin, glucose is not able to suppress glucagon release in vivo. However, the precise mechanism by which insulin suppresses glucagon secretion from alpha cells is unknown.

In this study, we show that insulin induces activation of GABA_A receptors in the alpha cells by receptor translocation via an Akt kinase-dependent pathway. This leads to membrane hyperpolarization in the alpha cells and, ultimately, suppression of glucagon secretion. We propose that defects in this pathway(s) contribute to diabetic hyperglycemia.

quate understanding of the mechanisms underlying suppression of glucagon by insulin in response to hyperglycemia.

Secretion of glucagon from α cells is regulated by various factors, including glucose, zinc, and the chemical transmitter γ -aminobutyric acid (GABA) (Pipeleers et al., 1985; Ishihara

(Rorsman et al., 1989). The failure to detect an increase in GABA release does not exclude the possibility that there is an increase in the responsiveness of GABA_ARs on α cells upon hyperglycemia; however a clear-cut mechanism has not been delineated.



GABA exerts protective and regenerative effects on islet beta cells and reverses diabetes

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Edited* by Roger H. Unger, Touchstone Center for Diabetes Research, Dallas, TX, and approved June 2, 2011 (received for review February 23, 2011)

Type 1 diabetes (T1D) is an autoimmune disease characterized by insulinitis and islet β -cell loss. Thus, an effective therapy may require β -cell restoration and immune suppression. Currently, there is no treatment that can achieve both goals efficiently. We report here that GABA exerts antidiabetic effects by acting on both the islet β -cells and immune system. Unlike in adult brain or islet α -cells in which GABA exerts hyperpolarizing effects, in islet β -cells, GABA

have demonstrated that β -cells also express GABA_ARs (20, 21), forming an autocrine GABA signaling system (20, 21). However, the role of this autocrine GABA signaling in the regulation of β -cell functions remains largely unknown.

It has been previously demonstrated that persistent high glucose or elevated cytoplasmic ATP levels could suppress GABA production and its release from β -cells (22). In view of the critical role

Daily GABA injections initiated 7 d before streptozotocin (STZ) treatment prevented β -cell loss. Thus, β -cell mass was preserved, whereas α -cell mass was reduced. Consistently, GABA-treated mice showed higher circulating insulin, lower glucagon, nearly normal glycemia, and improved metabolic conditions, and maintained close to normal glucose tolerance, during a period of 53 d after STZ injections.

In the adult brain, GABA induces a fast inhibition in neurons mainly through the GABA_A receptor (GABA_AR) (9). Activation of GABA_AR, a ligand-gated Cl⁻ ion channel, results in membrane hyperpolarization as a consequence of Cl⁻ influx (8). In the developing brain, however, activation of GABA_AR induces membrane depolarization, which regulates neuronal cell proliferation and maturation (10–12). GABA_ARs are also expressed in various immune cells, including T cells, and appear to exert immunoinhibitory effects (13–15).

GABA is produced by pancreatic β -cells (16). GABA released from β -cells can act on GABA_AR in the α -cells, causing membrane hyperpolarization and hence suppressing glucagon secretion (17, 18). An impaired insulin-Akt-GABA_AR-glucagon secretory pathway in the islet may be an underlying mechanism for unsuppressed glucagon secretion, despite hyperglycemia, in diabetic subjects (18, 19). Remarkably, studies by our group and others

Author contributions: N.S., G.J.P., and Q.W. designed research; N.S., H.Q., M.A., Y.G., F.Z., R.L., N.Z., R.C., T.N., H.Z., Z.-P.F., and Q.W. performed research; N.S., H.Q., M.A., Y.G., F.Z., R.L., Y.L., N.Z., R.C., T.N., T.J., H.Z., W.-Y.L., Z.-P.F., G.J.P., and Q.W. analyzed data; and N.S., G.J.P., and Q.W. wrote the paper.

Conflict of interest statement: A patent application authored by N.S. and Q.W. has been submitted for an invention related to this study.

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GABA Promotes Human β -Cell Proliferation and Modulates Glucose Homeostasis

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γ -Aminobutyric acid (GABA) exerts protective and regenerative effects on β -cells. GABA has been shown to be the major source of β -cell renewal in

GABA treatment increased grafted β -cell proliferation, while decreasing apoptosis, leading to enhanced β -cell mass. This was associated with increased circulating human insulin and reduced glucagon levels. Importantly, GABA administration lowered blood glucose levels and improved glucose excursion rates.

nals responsible for β -cell proliferation and survival. Our findings suggest that GABA regulates human β -cell mass and may be beneficial for the treatment of diabetes or improvement of islet transplantation.

Expanding β -cell mass by promoting β -cell regeneration is a major goal of diabetes therapy. β -Cell proliferation has

In the β -cells, GABA induces membrane depolarization and increases insulin secretion (9), while in the α -cells it induces membrane hyperpolarization and suppresses glucagon secretion (10). In mice, we previously observed that it enhanced β -cell proliferation and reduced β -cell death, which reversed T1D (9). Indeed, in various disease models, GABA exerts trophic effects on β -cells and

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Study of GABA in Healthy Volunteers: Pharmacokinetics and Pharmacodynamics

OPEN ACCESS

Junfeng Li^{1††}, Zhaoyun Zhang^{1††}, Xiaoxia Liu¹, Yi Wang¹, Fei Mao¹, Junjun Mao²,
Xiaolan Lu¹, Dongdong Jiang¹, Yun Mao¹, Jia Ying Lu², Guoping Cao¹, Jing Zhang¹

Gamma aminobutyric acid (GABA) exerts β -cell regenerative and immunoregulatory effects. Specifically, GABA stimulates β -cell replication, protects β -cells against apoptosis, and attenuates insulinitis. These effects result in an enhanced functional β -cell mass and, in mice, this can reverse disease

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times/day for 7 days, with a 7-day washout between each period. GABA was rapidly absorbed (T_{max} : 0.5 ~ 1 h) with the half-life ($t_{1/2}$) of 5 h. No accumulation was observed after repeated oral GABA administration for 7 days. Remarkably, GABA significantly increased circulating insulin levels in the subjects under either fasting (1.6-fold, single dose; 2.0-fold, repeated dose; $p < 0.01$) or fed conditions (1.4-fold, single dose; 1.6-fold, repeated dose; $p < 0.01$). GABA also increased glucagon levels only under fasting conditions (1.3-fold, single dose, $p < 0.05$; 1.5-fold, repeated dose, $p < 0.01$). However, there were no significant differences in the insulin-to-glucagon ratio and no significant change in glucose levels in these healthy subjects during the study period. Importantly, GABA significantly decreased glycated albumin levels in the repeated dosing period. Subjects with repeated dosing showed an elevated incidence of minor adverse events in comparison to placebo or the single dosing period, most notably transient discomforts such as dizziness and sore throat. However, there were no serious adverse events observed throughout the study. Our data show that GABA is rapidly absorbed and tolerated in human beings; its endocrine effects, exemplified by increasing islet hormonal secretion, suggest potential therapeutic benefits for diabetes.

Keywords: GABA, pharmacokinetics, glucagon, insulin, glycated albumin



Study of GABA in Healthy Volunteers: Pharmacokinetics and

Twelve subjects were subjected to an open-labeled, three-period trial involving sequential oral administration of placebo, 2 g GABA once, and 2 g GABA three times/day for 7 days, with a 7-day washout between each period. GABA was rapidly absorbed (T_{max} : 0.5 ~ 1 h) with the half-life ($t_{1/2}$) of 5 h.

GABA significantly increased circulating insulin levels in the subjects under either fasting or fed conditions. GABA also increased glucagon levels only under fasting conditions.

Importantly, GABA significantly decreased glycated albumin levels in the repeated dosing period.

STEP 3 – C-Peptide

If C-peptide is above 2.5:

1. Mitochondrial support

- * Glutathione
- * NAC
- * ALA
- * Carnitine

If C-peptide is below 1.1 + elevated A1C:

1. GABA

Insulin resistance is a cellular antioxidant defense mechanism

Kyle L. Hoehn^{a,1,2,3}, Adam B. Salmon^{b,1}, Cordula Hohnen-Behrens^a, Nigel Turner^a, Andrew J. Hoy^a, Ghassan J. Maghzal^c, Roland Stocker^c, Holly Van Remmen^b, Edward W. Kraegen^a, Greg J. Cooney^a, Arlan R. Richardson^b, and David E. James^{a,2}

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In the present study, we took a comprehensive approach to identify factor(s) that might unify multiple models of IR. Initially, we compared four diverse models of IR including chronic treatment with insulin, corticosteroids, proinflammatory cytokines, or lipid in both muscle and adipose cell lines.

We have now identified a direct correlation between mitochondrial oxidative stress in all models.

matory corticosteroids. Do such factors converge at a common intermediate in the insulin action pathway or does IR represent a collection of distinct cellular disorders? For example, endoplasmic reticulum (ER) stress, proinflammatory responses, oxidative stress, intracellular ceramide accumulation, or the activation of JNK, IKK, or PKC are all currently implicated in the development of IR in overnourished or obese rodents (2, 3). In such models, correcting any one of these intracellular stresses is sufficient to improve IR leading to the possibility that these factors are somehow interconnected. One view is that insulin receptor substrate 1 (IRS1) represents a common convergence point for many defects contributing to IR (4). However, this view has been challenged in that the ability of IRS1-independent receptor tyrosine kinases to activate

Author contributions: K.L.H., A.B.S., and D.E.J. designed research; K.L.H., A.B.S., C.H.-B., N.T., A.J.H., and G.J.M. performed research; K.L.H., A.B.S., R.S., H.V.R., E.W.K., G.J.C., and A.R.R. contributed new reagents/analytic tools; K.L.H., A.B.S., and C.H.-B. analyzed data; and K.L.H. and D.E.J. wrote the paper.

The authors declare no conflict of interest.

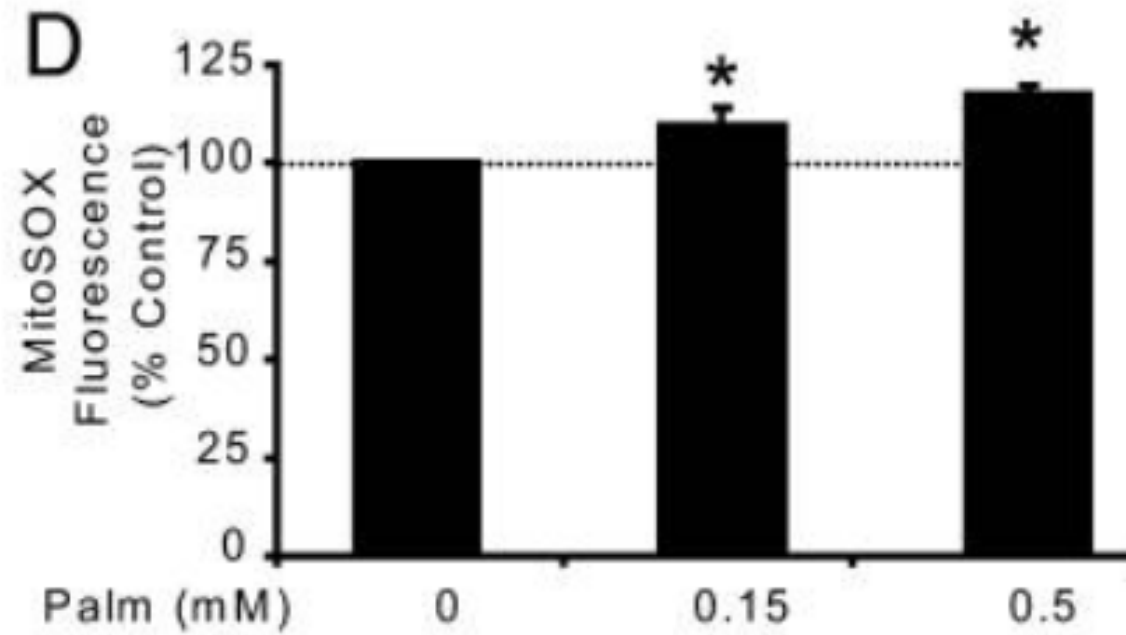
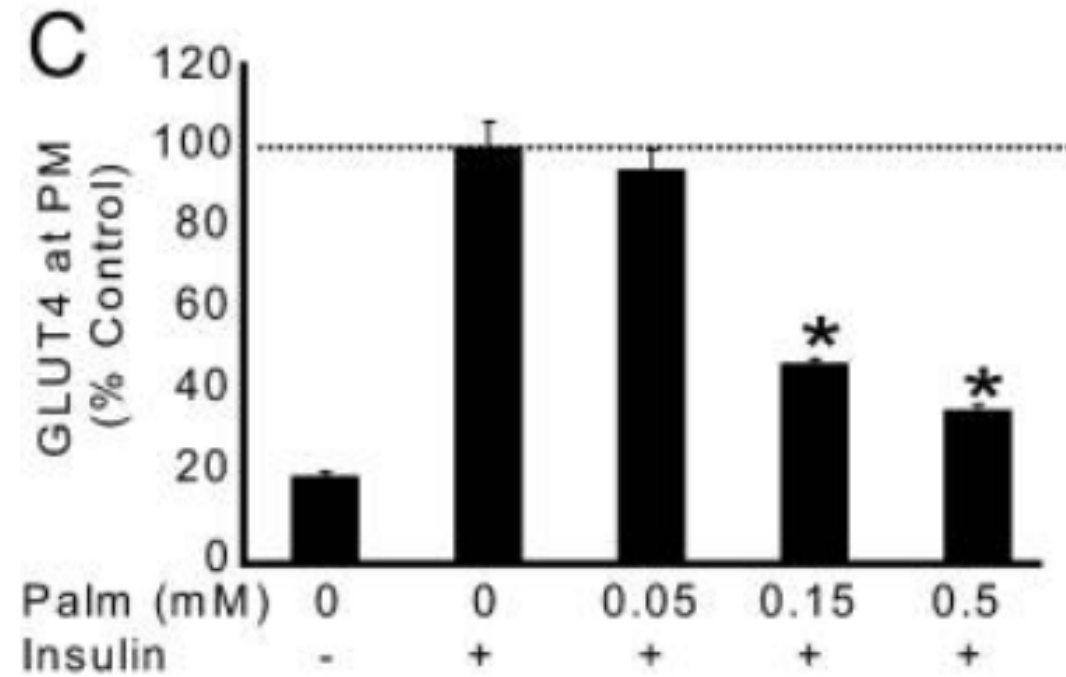
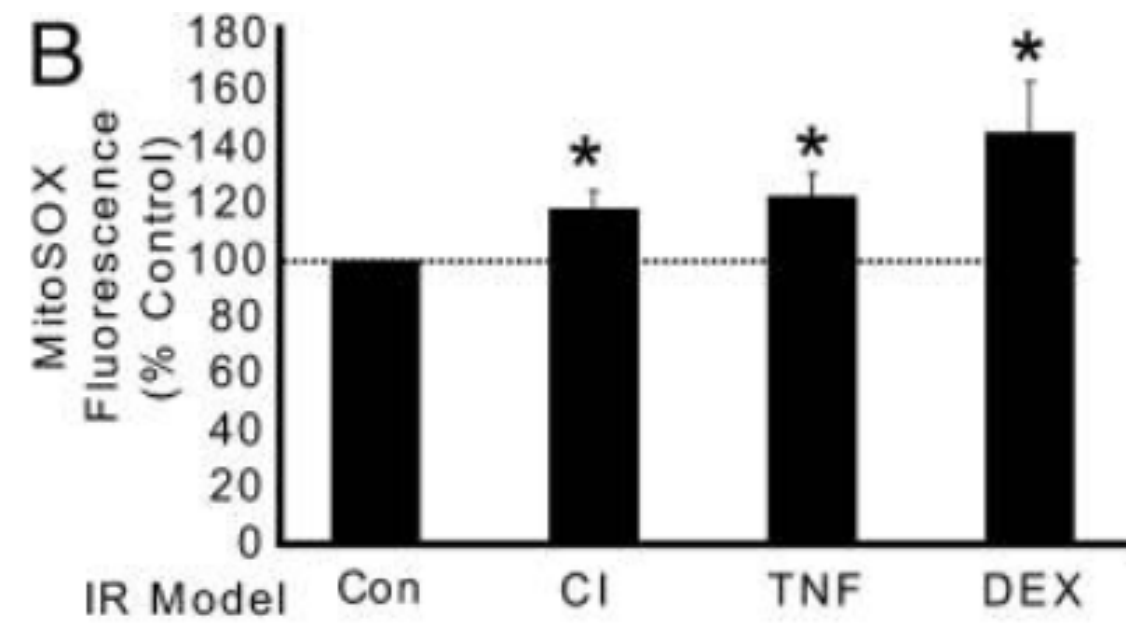
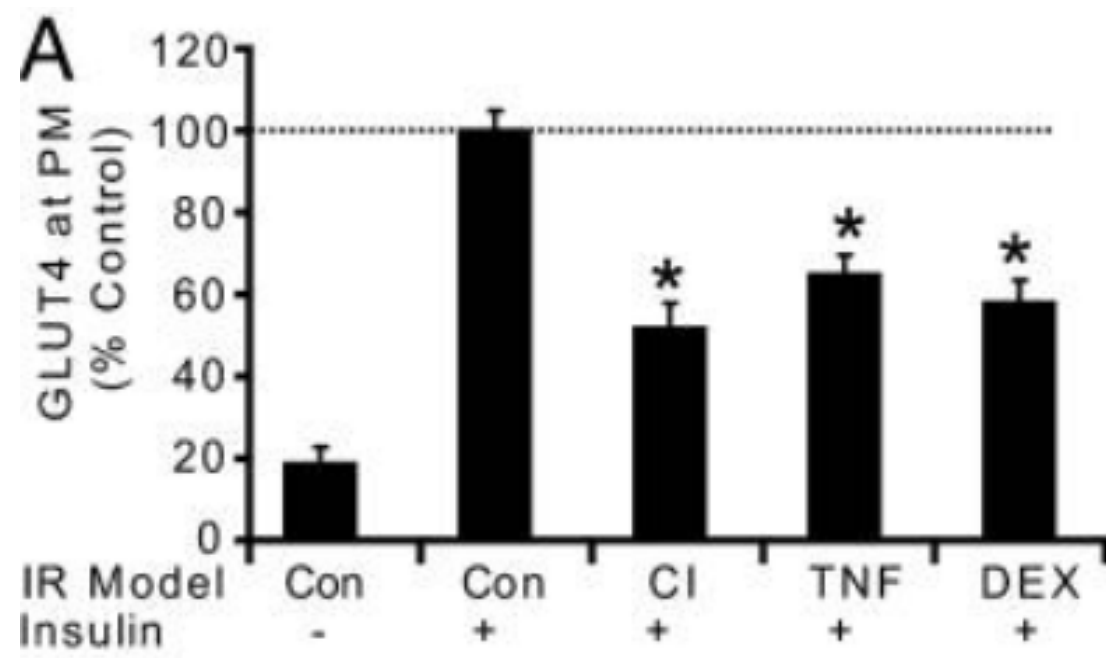
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Insulin resistance is a cellular antioxidant defense mechanism

Kyle L. Hoehn^{a,1,2,3}, Adam B. Salmon^{b,1}, Cordula Hohnen-Behrens^a, Nigel Turner^a, Andrew J. Hoy^a, Ghassan J. Maghzal^c, Roland Stocker^c, Holly Van Remmen^b, Edward W. Kraegen^a, Greg J. Cooney^a, Arlan R. Richardson^b, and David E. James^{a,2}

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Mitochondrial $O_2^{\bullet-}$ has previously been linked to hyperglycemia-induced metabolic dysfunction in endothelial cell systems and in inflammation in adipocytes.

A major advance of the present study is the observation that mitochondrial $O_2^{\bullet-}$ is upstream of IR in skeletal muscle and adipose tissue.

The origin of IR has been difficult to elucidate in part due to the diverse set of risk factors linked to this condition including over-nutrition, physical inactivity, pregnancy, Hepatitis C, polycystic ovarian syndrome, HIV protease inhibitor therapy, and antiinflammatory corticosteroids. Do such factors converge at a common intermediate in the insulin action pathway or does IR represent a collection of distinct cellular disorders? For example, endoplasmic reticulum (ER) stress, proinflammatory responses, oxidative stress, intracellular ceramide accumulation, or the activation of JNK, IKK, or PKC are all currently implicated in the development of IR in overnourished or obese rodents (2, 3). In such models, correcting any one of these intracellular stresses is sufficient to improve IR leading to the possibility that these factors are somehow interconnected. One view is that insulin receptor substrate 1 (IRS1) represents a common convergence point for many defects contributing to IR (4). However, this view has been challenged in that the ability of IRS1-independent receptor tyrosine kinases to activate

described a reproducible system for studying IR in myotubes and adipocytes in culture relying on the translocation of the facilitative glucose transporter GLUT4 to the plasma membrane (5). This

Author contributions: K.L.H., A.B.S., and D.E.J. designed research; K.L.H., A.B.S., C.H.-B., N.T., A.J.H., and G.J.M. performed research; K.L.H., A.B.S., R.S., H.V.R., E.W.K., G.J.C., and A.R.R. contributed new reagents/analytic tools; K.L.H., A.B.S., and C.H.-B. analyzed data; and K.L.H. and D.E.J. wrote the paper.

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This hypothesis is exciting for a number of reasons. For example, it suggests that IR may be a protective mechanism, in which case we should perhaps reconsider using therapeutic strategies to overcome unless they also eliminate the primary defect. Moreover, it suggests that cells have evolved sophisticated mechanisms to not only guard against nutrient lack, such as the AMPK pathway, but also nutrient excess.

The origin of IR has been difficult to elucidate in part due to the diverse set of risk factors linked to this condition including over-nutrition, physical inactivity, pregnancy, Hepatitis C, polycystic ovarian syndrome, HIV protease inhibitor therapy, and antiinflammatory corticosteroids. Do such factors converge at a common intermediate in the insulin action pathway or does IR represent a collection of distinct cellular disorders? For example, endoplasmic reticulum (ER) stress, proinflammatory responses, oxidative stress, intracellular ceramide accumulation, or the activation of JNK, IKK, or PKC are all currently implicated in the development of IR in overnourished or obese rodents (2, 3). In such models, correcting any one of these intracellular stresses is sufficient to improve IR leading to the possibility that these factors are somehow interconnected. One view is that insulin receptor substrate 1 (IRS1) represents a common convergence point for many defects contributing to IR (4). However, this view has been challenged in that the ability of IRS1-independent receptor tyrosine kinases to activate

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Insulin Resistance as a Physiological Defense Against Metabolic Stress: Implications for the Management of Subsets of Type 2 Diabetes

The β -cell is particularly vulnerable to glucolipotoxicity. Other tissues, such as heart and skeletal muscle, that express the insulin-regulated glucose transporter GLUT4 have the capacity to protect themselves from glucolipotoxicity by developing IR, which restrains glucose entry into cells and therefore the glucose arm of this potentially damaging process.

T2D. Potential molecular mechanisms underlying these concepts; their clinical implications for stratification of T2D management, particularly in overweight and obese patients with difficult glycemic control; and future research requirements are discussed.

high-dose insulin therapy) will cause them harm. We believe that the concept of “insulin-induced metabolic stress” provides a plausible explanation for many of the unexpected outcomes of major T2D clinical trials. The important implications of this concept for ongoing

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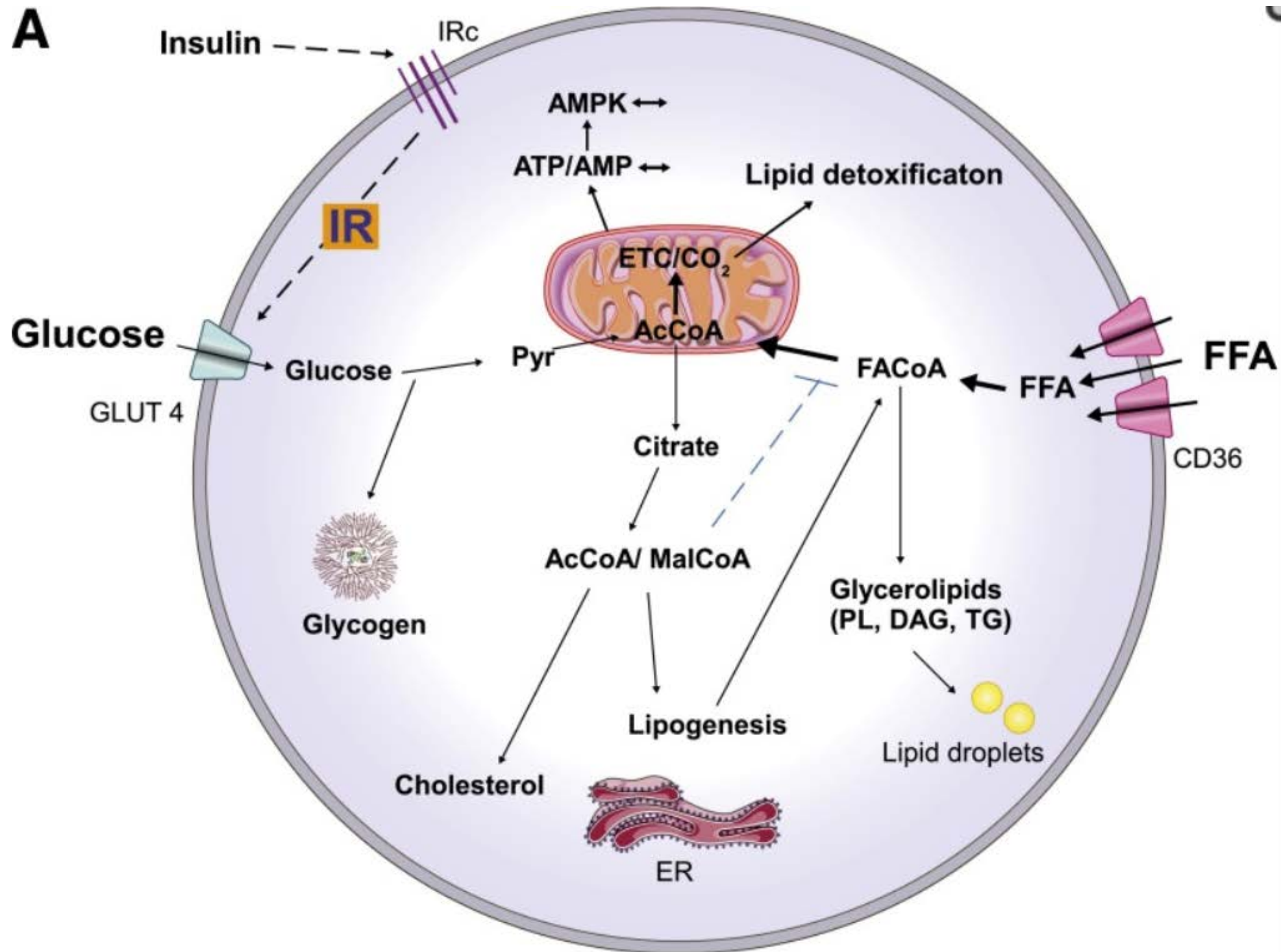
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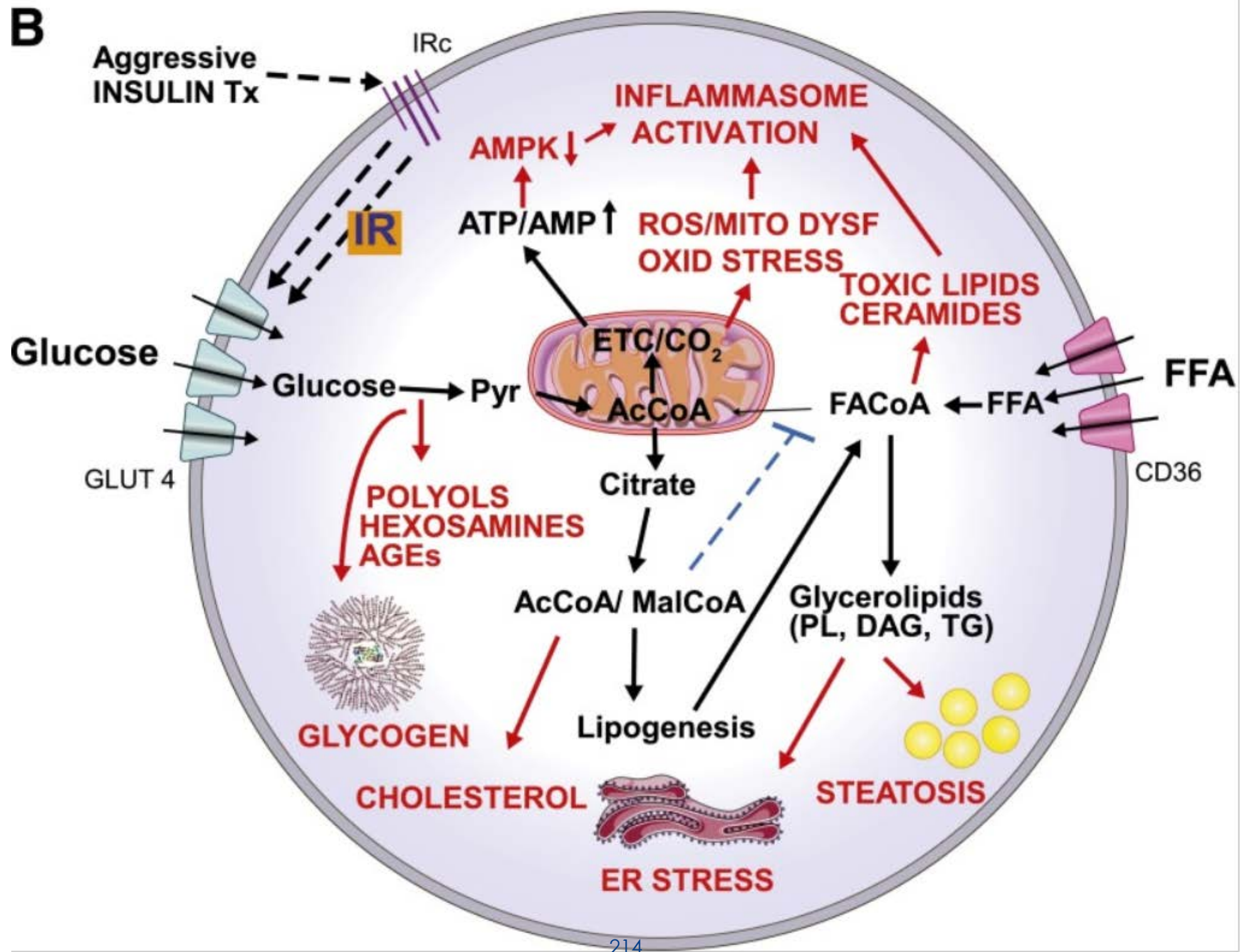


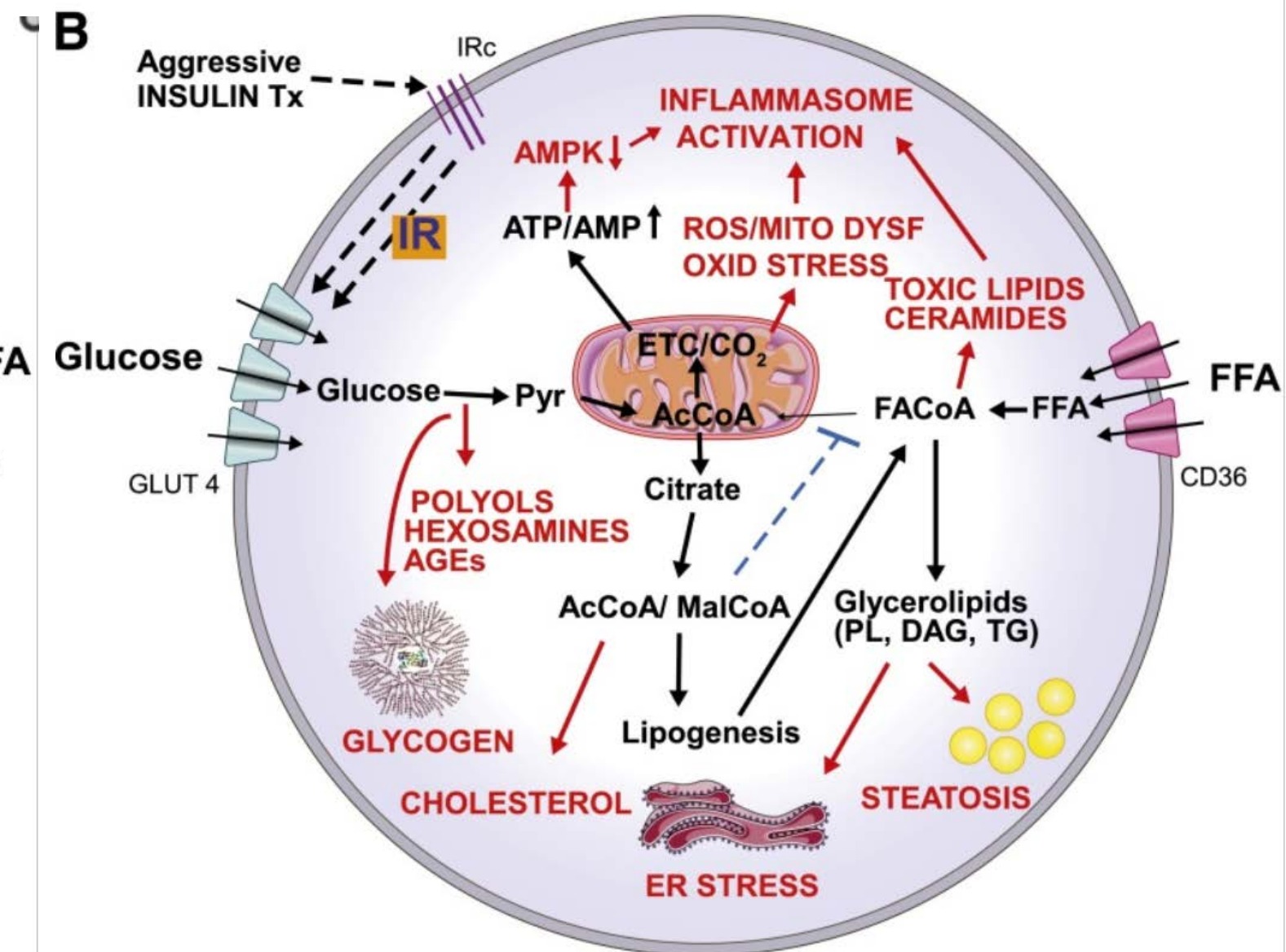
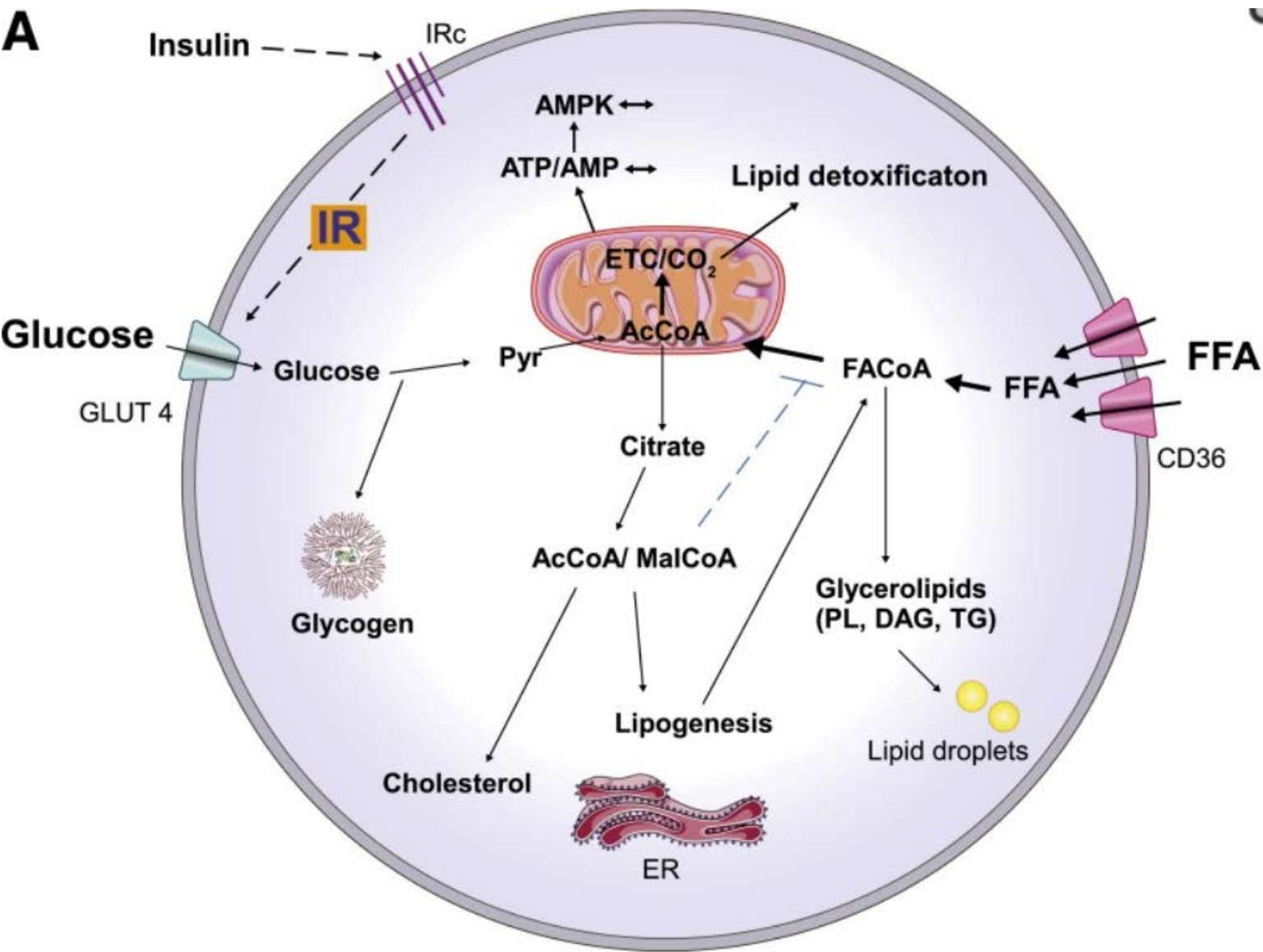
Insulin Resistance as a Physiological Defense Against

The combination of **high levels of glucose and FFA entry into cells will overload the electron transfer chain** with reducing equivalents resulting in mitochondrial dysfunction and increased reactive oxygen species (ROS) production. The increased glucose entry will also alter the malonyl-CoA/AMPK metabolic network to favor the partitioning of the FFA toward synthesis of complex lipids, including cholesterol and ceramide, and glucolipotoxicity, contributing to both mitochondrial dysfunction and endoplasmic reticulum stress.

The increased FFA levels will also impede glucose oxidation, particularly at the level of pyruvate dehydrogenase, such that glucose flux into pathways above this step, including glycogen synthesis, the polyol and hexosamine pathways, and the production of advanced glycation end product (AGE) precursors, are likely to be increased.







Review

Patient A

- * Low Glycomark
- * Elevated C-peptide
- * Normal A1C

Patient B

- * Normal Glycomark
- * Elevated C-peptide
- * Elevated A1C (5.8%)

Patient C

- * Normal Glycomark
- * Low C-peptide
- * Elevated A1C (5.8%)

Patient D

- * Low Glycomark
- * Elevated C-peptide
- * Normal A1C (5.3%)

- * Lipoic Acid - GLUT-4
- * Chromium - **insulin receptors**
- * Cinnamon - **insulin receptors**
- * Banaba leaf - decreases hepatic gluconeogenesis, **insulin-like glucose transport**, ppar-alpha (liver), ppar-gamma (adipose)
- * Berberine - AMPK, **insulin receptors**, inducing glycolysis, promoting glucagon peptide-1 release, inhibiting DPP-4, inhibiting hepatic gluconeogenesis
- * Myricetin - IRS-1, PI3-Kinase, GLUT 4 (Post receptor insulin signaling)
- * Gymnema - reducing glucose absorption, regenerates beta cells, **endogenous insulin production**
- * Fish oil - insulin sensitivity in skeletal muscle
- * Nopal - high soluble fiber and pectin, intestinal glucose absorption,
- * Salacia Oblong - alpha-glucosidase inhibitor, ppar-alpha
- * Green Tea - upregulates glycogenesis, downregulate glycogenolysis, protects against glycation,
- * Bilberry - **insulin sensitivity**
- * American Ginseng - **insulin sensitivity**
- * Glucomannan - water-soluble dietary fiber, glucose absorption
- * Bitter Melon - structurally similar to insulin
- * Vanadium - mimics insulin
- * Holy Basil - increases insulin secretion
- * Fenugreek - stimulates insulin secretion

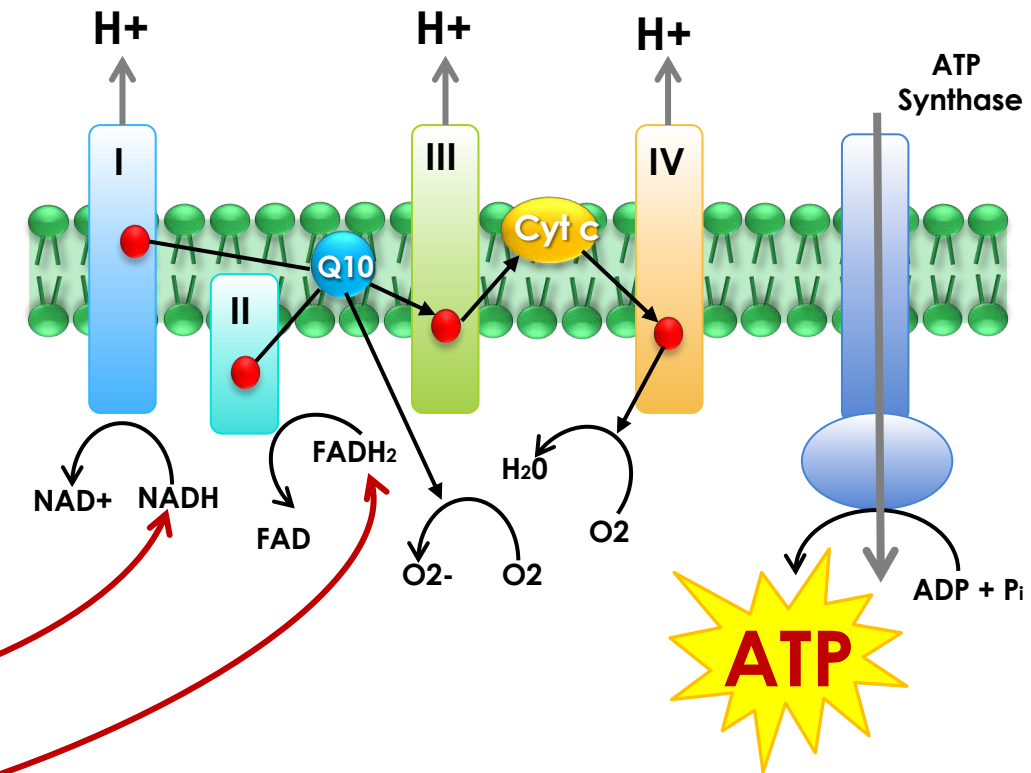
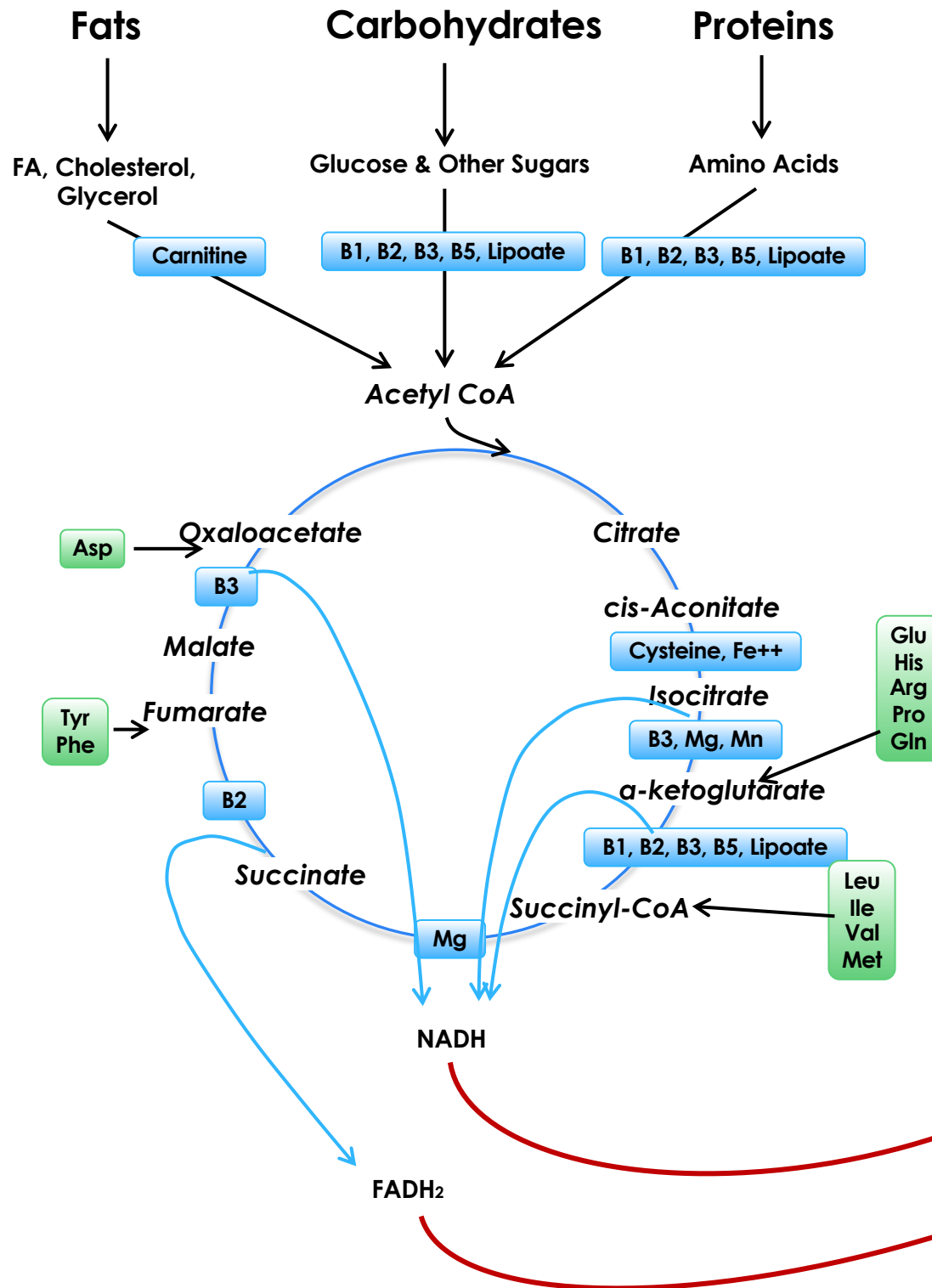
	Hypoglycemia	Early IR	Insulin Resistance	Late IR	Type II Diabetes
Glucose	↓	Normal	↑	↑↑	↑↑↑
C-Peptide	↑/N	↑	↑↑	Normal	↑/↓
HA1C	Normal/Low	Normal	↑	↑↑	↑↑↑
Cholesterol/ Triglyceride	Normal	Normal	Less than 2:1	Less than 2:1	Less than 2:1
LDH	↓	?	?	?	?

What have you learned so far?

Section Break Number 3

CBC with Differential

Nutrient Deficiency



ETC complexes: CoQ10, copper, iron, riboflavin
 Ox-Phos enzymes: MnSOD, Cu/Zn SOD, GPX
 Ox-Phos nutrients: Mn, SOD, Cu, Zn, selenium, glutathione

CBC

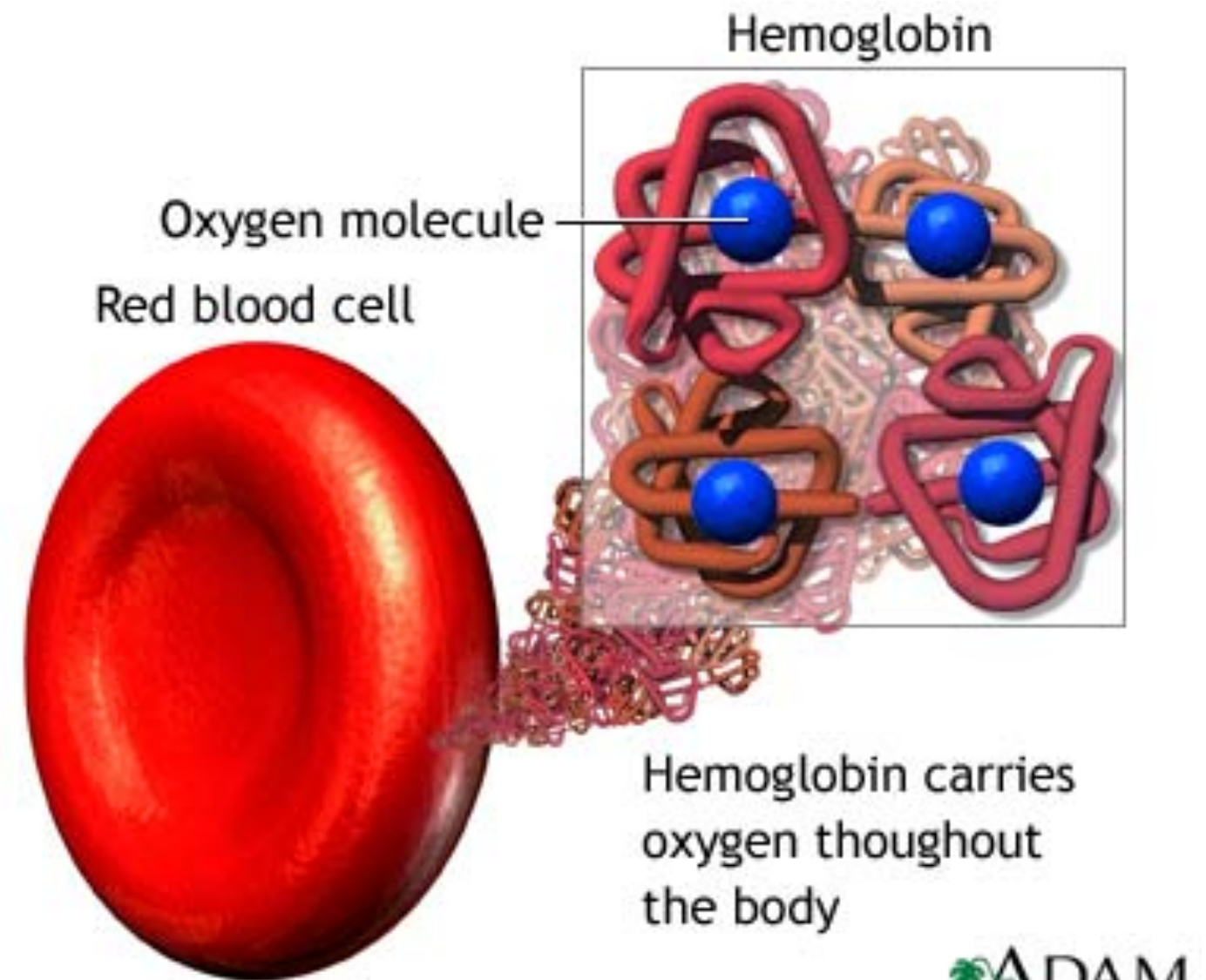
- * Red blood cells (count)
- * Hemoglobin
- * Hematocrit
- * Mean Corpuscular Volume (MCV)
- * Mean Corpuscular Hemoglobin (MCH)
- * Mean Corpuscular Hemoglobin Concentration (MCHC)
- * Red Cell Distribution Width
- * White blood cell (count)
- * Platelet (count)

CBC with differential

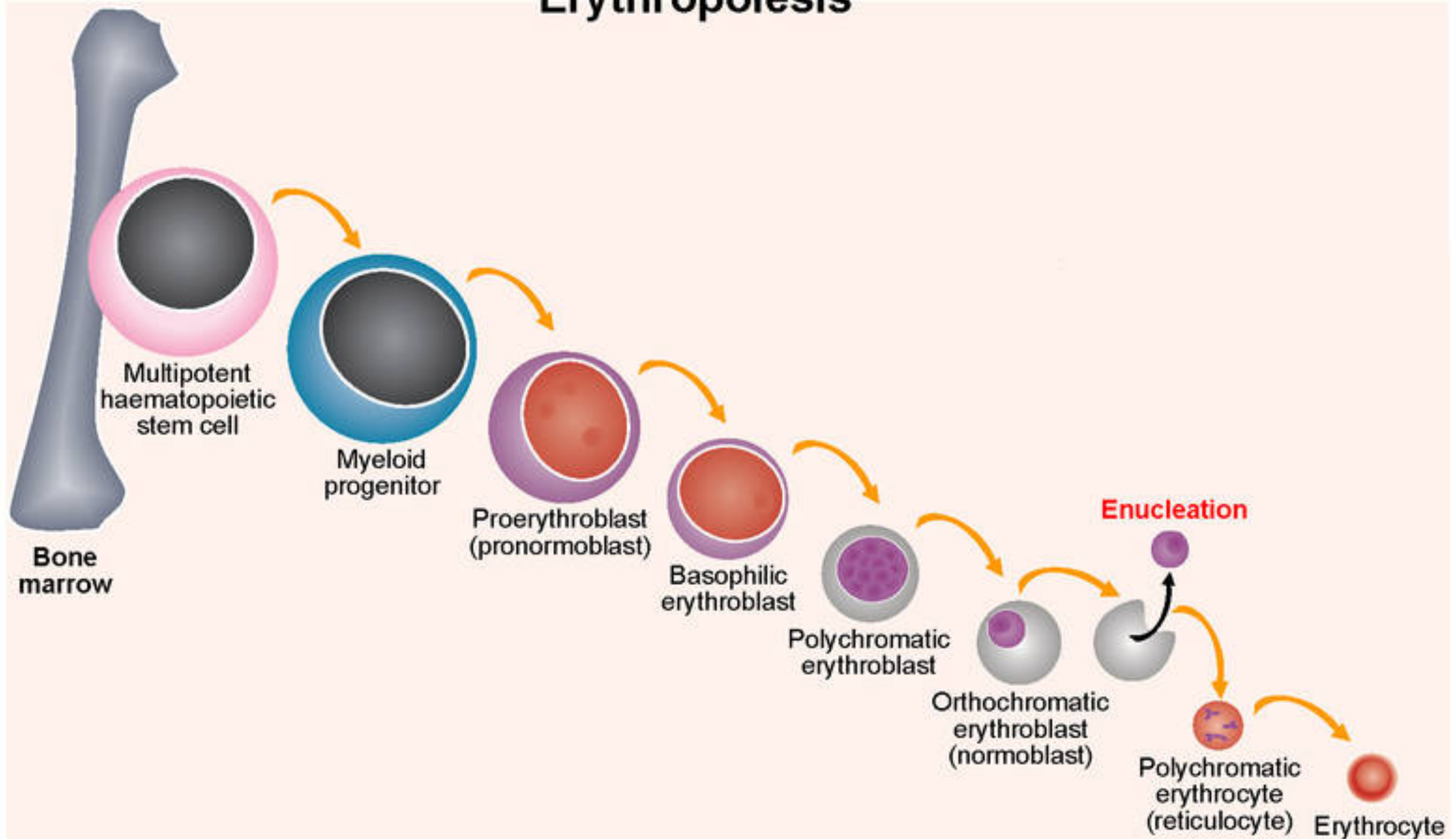
- * Includes the markers of a CBC
- * Neutrophils
- * Lymphocytes
- * Monocytes
- * Eosinophils
- * Basophils

Red Blood Cells

- * Red blood cells are anucleated cells, produced in bone marrow and have a life span of approximately 120 days.
- * They have a phospholipid membrane
- * Contain 280 *million* molecules of hemoglobin
- * Contain cytosol and enzymes (PPP and glycolysis)



Erythropoiesis



The development of erythrocytes in bone marrow is regulated by the hormone erythropoietin that stimulates the differentiation of progenitor cells into erythroid precursor cells. A number of intermediate cell stages can be identified beginning with the proerythroblast (pronormoblast). The cell nucleus is extruded at the orthochromatic erythroblast (normoblast) stage before formation of an enucleated polychromatic erythrocyte (reticulocyte). These cells are released into the circulation where they mature into functional erythrocytes.



Red Blood Cell

- * In the bone marrow, the hormones erythropoietin (kidneys) and testosterone can stimulate RBC production
- * RBCs are broken down by macrophages in the spleen, but can also be catabolized by the liver as well

Red Blood Cell count

- * Number of red blood corpuscles per volume of blood

Traditional Reference Range:

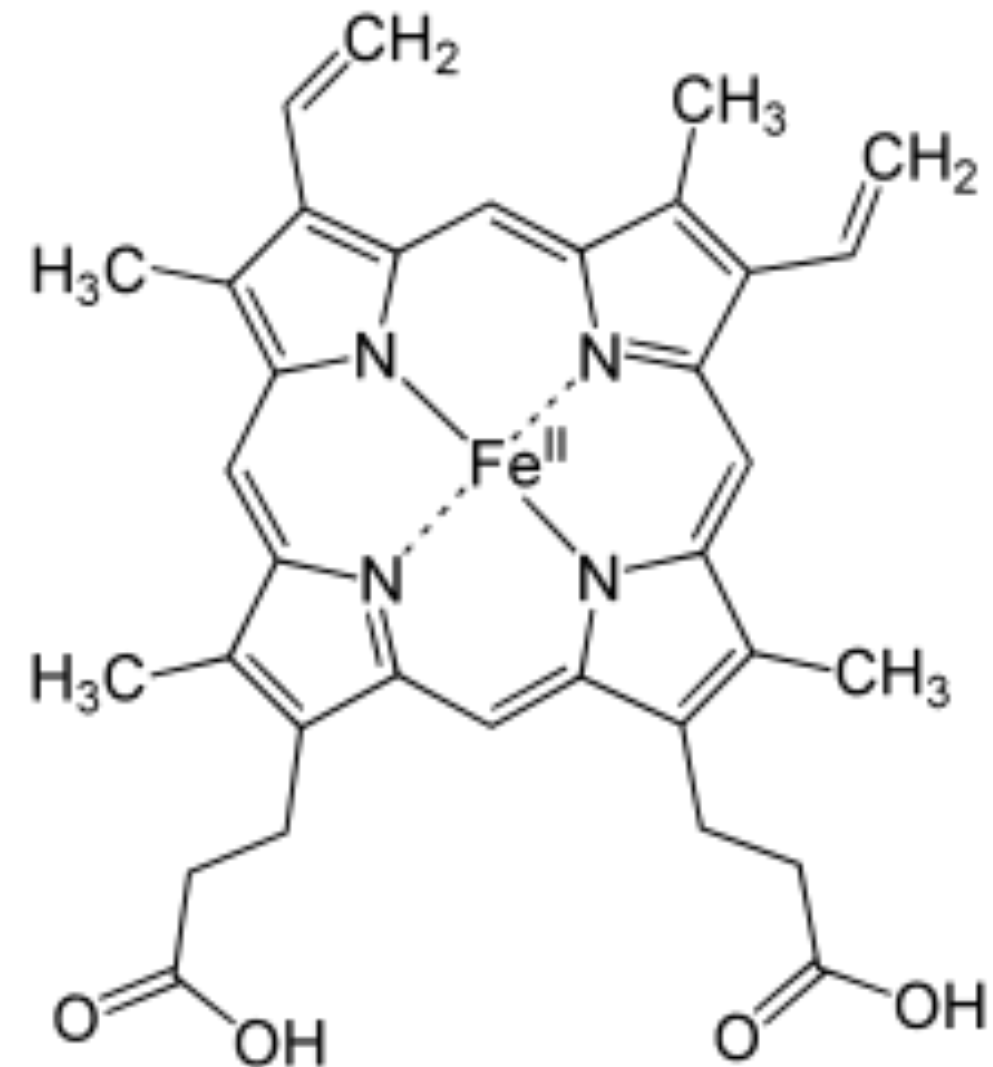
- * Male: $4.5-5.9 \times 10^6$ cells/uL
- * Female: $4.1-5.1 \times 10^6$ cells/uL

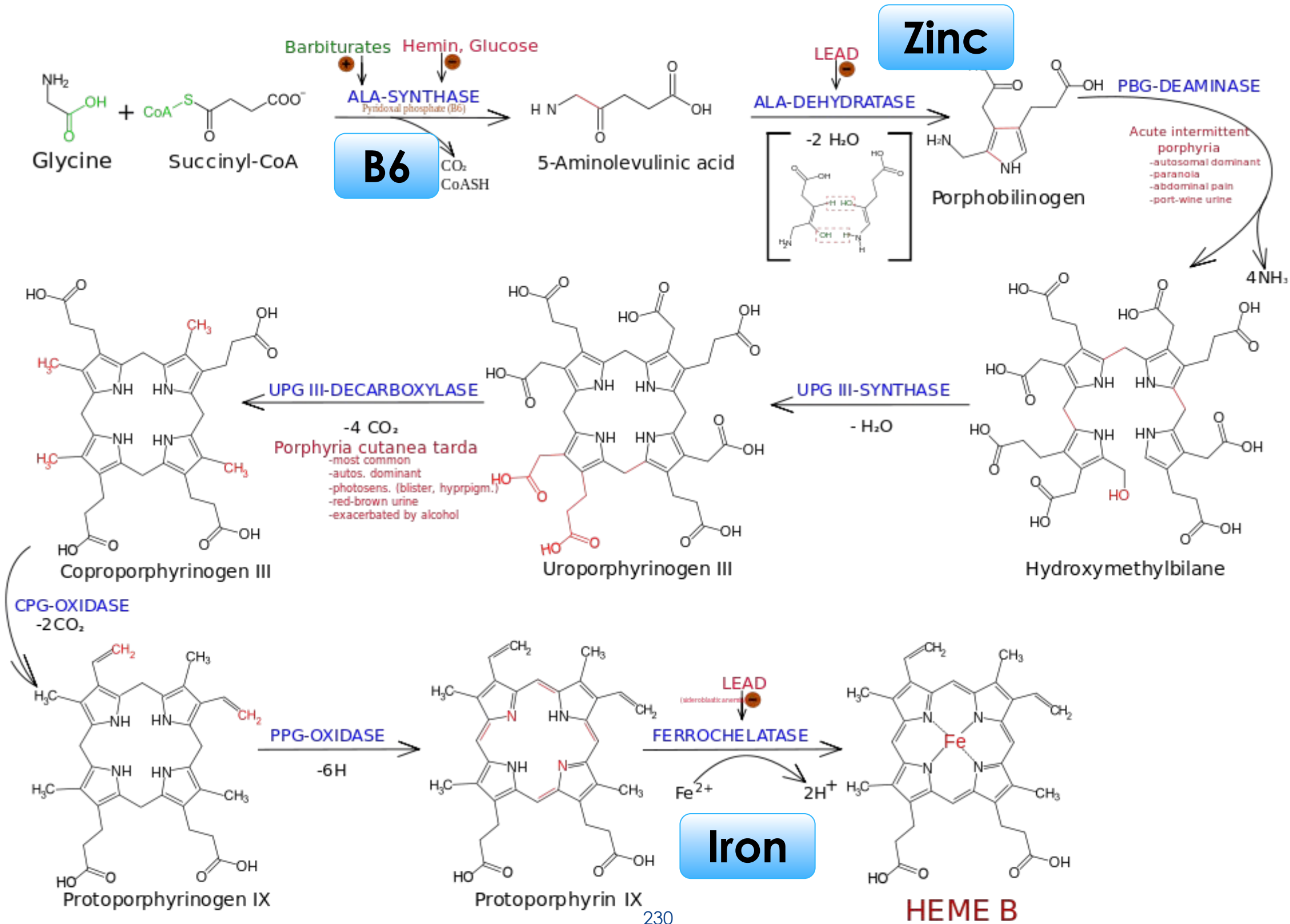
Optimal Reference Range:

- * Male: $4.8-5.5 \times 10^6$ cells/uL
- * Female: $4.4-4.8 \times 10^6$ cells/uL

Hemoglobin

- * Hemoglobin is the oxygen carrying component of blood
- * Requires protein, vitamin B6, zinc and iron (along with copper and vitamin C) for proper production
 - * A decrease of any of these nutrients can lead to poor hemoglobin production and smaller red blood cells
- * Also acts as important buffer of blood





Hemoglobin

- * Hemoglobin measurement is the amount of hemoglobin per unit of blood.

Traditional Reference Range:

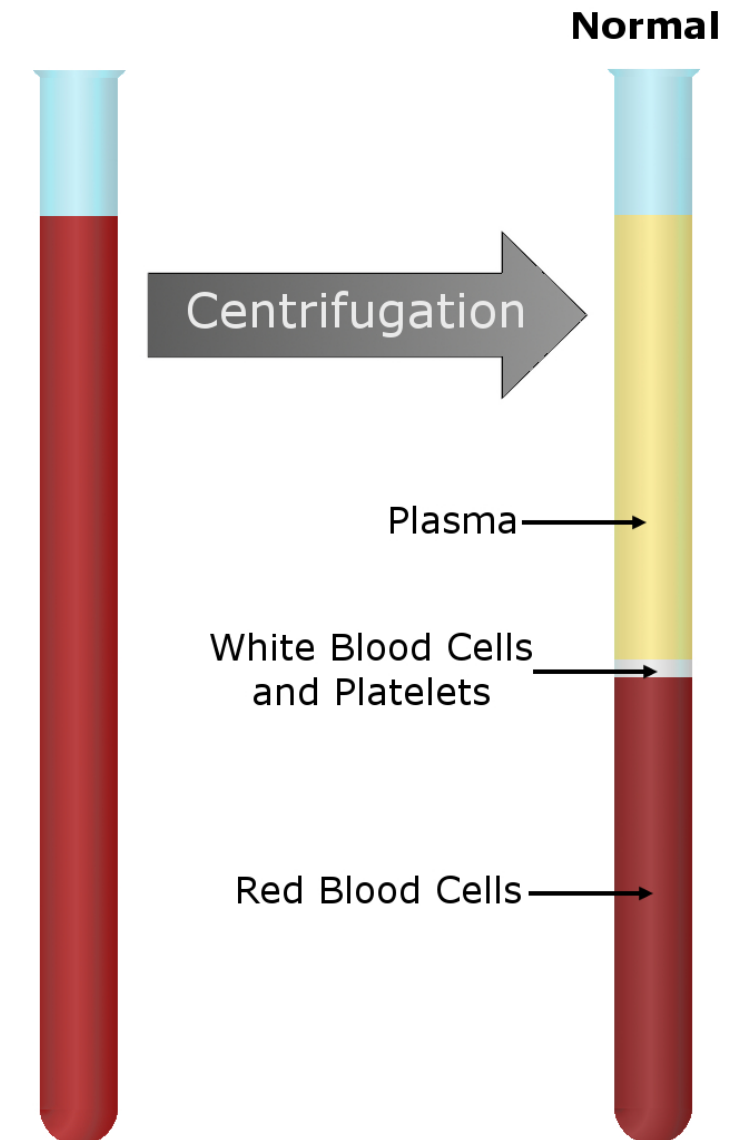
- * Men: 14.0-17.4 g/dL
- * Women: 12.0-16.0 g/dL

Optimal Reference Range

- * Men: 14.5-16 g/dL
- * Women: 13-14.5 g/dL

Hematocrit (Packed Cell Volume)

- * Hematocrit means “to separate blood”.
- * Percentage of volume of blood composed of red blood cells
- * Usually about three times the value of hemoglobin
 - * Size and shape of RBC can alter this



Hematocrit

Traditional Reference Range

- * Men: 42-52% or 0.42-0.52
- * Women: 36-48% or 0.36-0.48

Optimal Reference Range

- * Men: 44-49%
- * Women: 39-45%

***Hematocrit can be misleading. For example, small RBC can be more tightly packed and thus create a lower hematocrit. On the other hand, large red blood cells may not pack as well creating a falsely normal hematocrit despite having anemia.

Fewer, large RBC = normal hematocrit

RBC, Hemoglobin, Hematocrit - Elevated

Cause	Reason	Additional Inquiry
Dehydration	RBC can appear elevated due to hemoconcentration, or a decrease in plasma volume.	Evaluate other dehydration markers (albumin, BUN, Na/K, specific gravity).
Elevated testosterone	Testosterone can increase RBC production.	Evaluate testosterone signs, symptoms and markers.
Poor oxygenation	High altitude, smoking, poor lung function (eg asthma) could create relative hypoxia (low oxygen) in the body and increase RBC production to compensate.	Client history.
Thiamin deficiency	In a rat study, thiamine deficiency increased EPO and red blood cell production (past 30 days).	Evaluate other thiamin deficiency markers, including CO2 and anion gap (low CO2, increased anion gap).
Insulin resistance	Insulin has been found to regulate erythropoiesis. Increased insulin has been shown to increase RBC production.	Evaluate markers of glucose regulation.

RBC, Hemoglobin, Hematocrit - Decreased

Cause	Reason	Additional Inquiry
Anemia	Production, destruction or loss of RBC.	Evaluate other CBC markers.
Liver disease	Dilution due to volume overload, shortened RBC survival, poor response by bone marrow.	Evaluate liver markers.
Hypothyroidism	Decreased nutrient absorption and/or decreased EPO production.	Evaluate thyroid markers (free T3).
Exercise	Endurance exercise, during intense training, has been shown to decrease hemoglobin 3-8%.	Client history.
Arginine deficiency	Arginine deficiency has been shown to decrease hematopoietic stem cell differentiation and proliferation.	Nutritional history (including excess lysine).
Protein deficiency	Decreases EPO production.	Evaluate diet history.
Inflammation	Infection, inflammation, cytokine dysregulation. Increased destruction and/or decreased production (TNF α , IL-6, INF γ). 235	Evaluate markers of infection and infection.

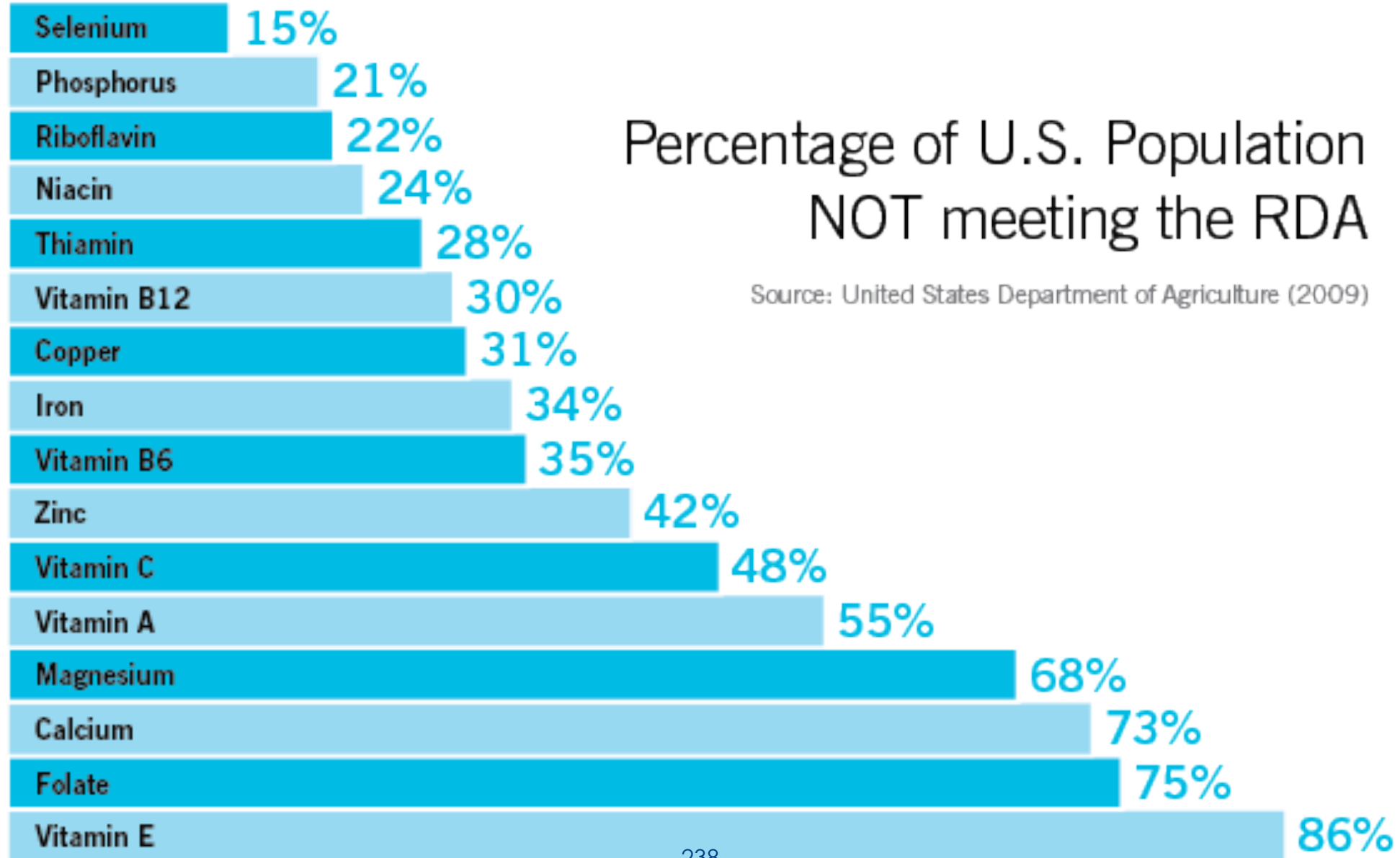
Infections Known To Cause Hemolytic Anemia

- * *Aspergillus*
- * *Bacillus anthracis*
- * *Babesia microti* and *Babesia divergens*
- * *Bartonella bacilliformis*
- * *Campylobacter jejuni*
- * *Clostridium perfringens*(*Welchii*)
- * *Coxsackievirus*
- * *Cytomegalovirus*
- * *Diplococcus pneumoniae*
- * *Epstein-Barr virus*
- * *Escherichia coli*
- * *Fusobacterium necrophorum*
- * *Haemophilus influenzae*
- * *Hepatitis A*
- * *Hepatitis B*
- * *Hepatitis C*
- * *Herpes simplex virus*
- * *Human immunodeficiency virus*
- * *Influenza A virus*
- * *Leishmania donovani*
- * *Leptospira interrogans* serovar *ballum* and/or *Leptospira kirschneri* serovar *butembo*
- * *Mumps virus*
- * *Mycobacterium tuberculosis*
- * *Mycoplasma pneumoniae*
- * *Neisseria intracellularis*
- * *Parvovirus B19*
- * *Plasmodium falciparum*
- * *Plasmodium malariae*
- * *Plasmodium vivax*
- * *Rubella virus*
- * *Rubeola virus*
- * *Salmonella*
- * *Shigella*
- * *Streptococcus*
- * *Toxoplasma*
- * *Trypanosoma brucei*
- * *Varicella virus*
- * *Vibrio cholerae*
- * *Yersinia enterocolitica*

RBC, Hemoglobin, Hematocrit - Decreased

Cause	Reason	Additional Inquiry
Vitamin E deficiency	Not used in human erythropoiesis, except when following a low calorie and/or low protein diet.	Evaluate nutritional history.
Magnesium deficiency	Causes increased iron absorption, uptake and storage, but appears to increase RBC fragility.	Evaluate magnesium status (high-normal serum iron).
Zinc deficiency	Causes poor hemoglobin synthesis, as well as increased RBC fragility (hemolysis).	Evaluate diet history as well as other zinc deficiency symptoms and markers (eg alkaline phosphatase).
Copper deficiency	Copper is required to keep iron in its usable (ferric) state. Thus low copper leads to microcytic hypochromic anemia, which is unresponsive to iron.	Low ceruloplasmin and/or serum copper. Possibly low neutrophils.
Selenium deficiency	Selenium mitigates oxidative stress. Leads to defective RBC differentiation, increased fragility, etc.	Evaluate diet history.
Vitamin B6 deficiency	Necessary to synthesize hemoglobin. Leads to microcytic anemia (sideroblastic).	Alcohol consumption. Diet history. Other vitamin B6 markers (low AST and/or ALT).
Vitamin A deficiency	Because iron and ferritin are likely normal, appears to be a iron transport issue. Also involved with EPO.	Diet history. Symptoms of vitamin A deficiency (night blindness).

How Deficient Are People?



Red Blood Cells – Interfering factors

Interfering Factors	
Elevated	Decreased
Stress	Recumbent during blood draw
Pregnancy	
Poor venipuncture (venous stasis)	

Combined Issues – The Art of Blood Chemistry Analysis

- * Influences to raise RCB count (dehydration, high testosterone, insulin resistance) combined with influences to decrease RBC count (anemia, hypothyroidism), can result in a normal RBC on a blood chemistry.
- * Similarly, influences to raise hematocrit (macrocytosis, dehydration) combined with influences to lower hematocrit (anemia), can result in normal hematocrit on a blood chemistry.



Mean Corpuscular Volume (MCV)

- * Estimate of the average RBC size (volume) measured in micrometers (femtoliter, fL)
- * Most clinically useful (versus MCH or MCHC)
- * Calculation

$$\text{MCV} = \text{Hct (\%)} \times 10 / \text{RBC}$$

- * Elevated MCV indicates larger than normal RBCs and decreased MCV indicates smaller than normal RBCs

Mean Corpuscular Volume (MCV)

Traditional Reference Range

- * 80-96 fL/cell

Optimal Reference Range

- * 84 – 92 fL/cell

Mean Corpuscular Volume (MCV)

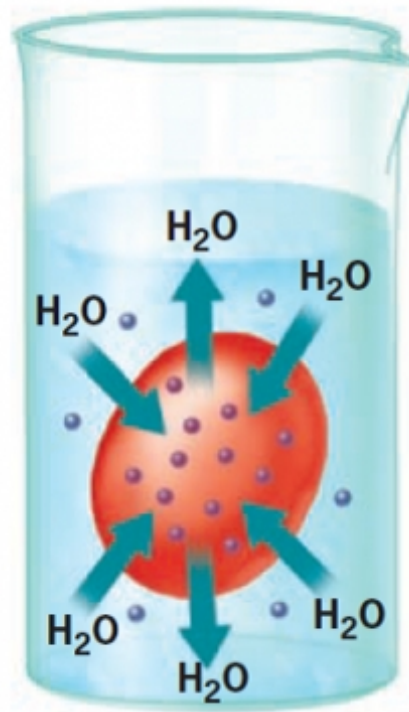
Interfering Factors	
Elevated	Decreased
Hyperglycemia	
Increased reticulocytes	
Increased WBC count	

- * It is also critical to understand that mixed red blood cells – some large, some small – can lead to a normal MCV*

Mean Corpuscular Volume (MCV)

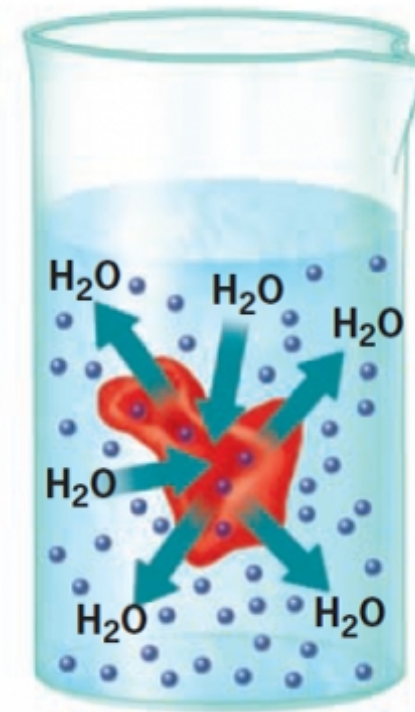
- * Elevated reticulocytes (large RBCs) can falsely elevate this value. Thus reticulocyte count is a highly valuable marker.
- * With reticulocyte count, one can also calculate RBC lifespan
- * Hyperglycemia can also falsely raise MCV.
- * When blood is mixed with a diluting solution (hypotonic) and there is high glucose, RBCs swell.

(a) Hypotonic solution



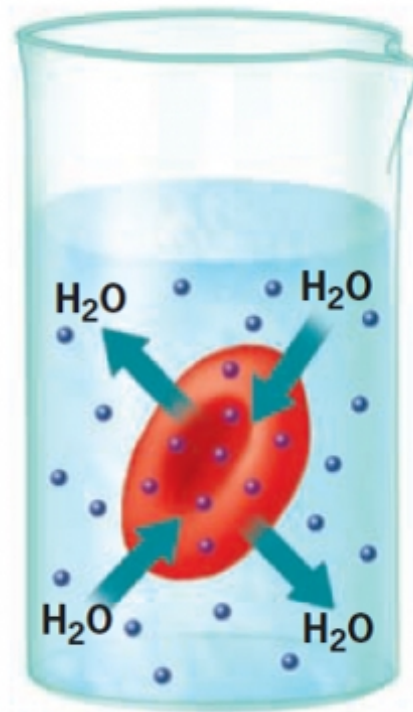
Net water gain
Cell swells

(b) Hypertonic solution



Net water loss
Cell shrinks

(c) Isotonic solution



No net loss or gain

Mean Corpuscular Hemoglobin (MCH)

- * The mean corpuscular hemoglobin is a measure of the average weight of hemoglobin per RBC.
- * It is calculated by:

$$\text{MCH} = \text{hemoglobin (g/dL)} \times 10 / \text{red blood cell count}$$

Mean Corpuscular Hemoglobin (MCH)

Traditional Reference Range

- * 26-34 pg/cell

Optimal Reference Range

- * 28-32 pg/cell

Mean Corpuscular Hemoglobin Concentration (MCHC)

- * Measures the average concentration of hemoglobin in the red blood cells.
- * Good at monitoring therapy as it uses hemoglobin and hematocrit in its calculation

$$\text{MCHC} = \text{hemoglobin (g/dL)} \times 100 / \text{hematocrit (\%)}$$

Mean Corpuscular Hemoglobin Concentration (MCHC)

Traditional Reference Range

- * 32-36 g/dL

Optimal Reference Range

- * 33-35 g/dL

MCH and MCHC

- * Hyperlipidemia (high cholesterol and/or triglyceride) can falsely increase MCH and MCHC. Therefore if elevated, quickly check lipid levels.

MCV, MCH, MCHC – Elevated - Macrocytic

Cause	Reason	Additional Inquiry
B12 and/or folate deficiency	Poor intake (vegetarians), impaired absorption (intrinsic factor, HCL), parasites, increased requirements (hyperthyroidism, pregnancy), enzyme deficiencies (MTHFR), poor protein binding.	Diet history. Other markers including genetic markers, MMA, homocysteine.
Vitamin C deficiency	Strong relationship with RBC folate.*	Diet history and other vitamin C symptoms and markers (alk phos, bleeding gums)
Alcohol	Result from nutritional deficiencies, chronic GI bleeding, hepatic dysfunction, or direct toxic effects of alcohol on erythropoiesis.	History.
Thiamin deficiency	Causes marked macrocytosis, likely due to decrease in nucleic acid synthesis (PPP).	Evaluate other thiamin deficiency markers, including anion gap.

*Consider adding vitamin C when supplementing with natural folate.

Vitamin C-related nutrient–nutrient and nutrient–gene interactions that modify folate status

Mark Lucock · Zoë Yates · Lyndell Boyd · Charlotte Naylor ·
Jeong-Hwa Choi · Xiaowei Ng · Virginia Skinner · Ron Wai ·
Jeremy Kho · Sa Tang · Paul Roach · Martin Veysey

Synthetic PteGlu correlates best with red cell folate at higher levels of intake

Natural 5CH(3)-H(4)-PteGlu(n) correlates best with red cell folate at lower levels of intake.

However, dietary vitamin C and 5CH(3)-H(4)-PteGlu(n) interact synergistically to correlate with red cell folate at higher levels of intake. No interaction between dietary vitamin C and PteGlu was observed.

This 'natural' nutrient-nutrient interaction may provide an alternative to synthetic PteGlu supplementation that is now linked to adverse phenomena/health outcomes. On its own, vitamin C also correlates with red cell folate and is strongly influenced by genetic variation in TS, MTHFR and MSR, genes critical for DNA and methionine biosynthesis that underpin erythropoiesis. Similarly, dietary vitamin C and 5CH(3)-H(4)-PteGlu(n) act synergistically to modify red cell folate status according to variation in folate genes: of note, heterozygosity for 2R3R-TS, SHMT, and all three MTHFR SNPs (G1793A, C677T and A1298C variants, respectively) promote a significant association with red cell folate.

MCV, MCH, MCHC – Normal - Normocytic

Cause	Reason	Additional Inquiry
Hemolytic anemia	Excess breakdown. Normal RBC production, but less RBC, hemoglobin, hematocrit	Normal RDW can help confirm hemolytic anemia. Also increased K and or Mg.
Kidney dysfunction	Decreased EPO production.	Kidney markers (BUN, creatinine, electrolytes, cystatin-C, urinalysis)
Anemia of Inflammation	Poor red blood cell production, but those that are created are normal size and color.	Additional markers such as transferrin binding receptor can differentiate anemia of inflammation. Also CRP, ESR, acute phase reactants
Vitamin E deficiency	Not used in human erythropoiesis, except when following a low calorie and/or low protein diet.	Evaluate nutritional history.
Magnesium deficiency	Causes increased iron absorption, uptake and storage, but appeared to increase RBC fragility.	Evaluate magnesium status (high-normal serum iron).
Selenium deficiency	Selenium mitigates oxidative stress. Leads to defective RBC differentiation, increased fragility, etc.	Evaluate diet history.
Oxidative stress	ROS suppress EPO synthesis.	Evaluate markers of oxidative stress (bilirubin, uric acid, GGT)
Vitamin C deficiency	Scurvy often results in normocytic anemia. Reduces vitamin E (lipid peroxidation), increased ROS, etc.	Diet history and other vitamin C symptoms and markers (alk phos, bleeding gums)

MCV, **MCH**, **MCHC** – Decreased - Microcytic

Cause	Reason	Additional Inquiry
Iron deficiency	Poor intake, malabsorption, increased iron loss (bleeding), increased iron requirements	Ferritin, TIBC, transferrin binding receptor.
Vitamin B6 deficiency	Necessary to synthesize hemoglobin. Leads to microcytic anemia (sideroblastic).	Alcohol consumption. Diet history. Other vitamin B6 markers (low AST and/or ALT).
Copper deficiency	Copper is required for converting iron into its useable form for transportation and heme synthesis.	Low ceruloplasmin and/or serum copper. Possibly low neutrophils. High zinc intake.
Zinc deficiency	Causes poor hemoglobin synthesis, as well as increased RBC fragility (hemolysis).	Diet journal. Decreased taste acuity, low hormones (testosterone), skin issues all can indicate zinc deficiency. Zinc tally test. Low alkaline phosphatase.
Vitamin A deficiency	Because iron and ferritin are likely normal, appears to be a iron transport issue. Also involved with EPO.	Diet history. Symptoms of vitamin A deficiency (night blindness).
Vitamin C deficiency	Interactions with iron.	Diet history and other vitamin C symptoms and markers (alk phos, bleeding gums)

Nutrient Deficiencies – Physical Manifestations

Vitamin A

- Acne, dry skin, rough (bumpy) skin on back of arms
- Photophobia, poor night vision, loss of “shininess” of eyes
- Gingivitis

Nutrient Deficiencies – Physical Manifestations

Vitamin B6

- Angular stomatitis/cheilosis, sore reddened tongue,
- Peripheral neuropathy, tremors/convulsions, behavioral disturbances (depression, slow mentation)

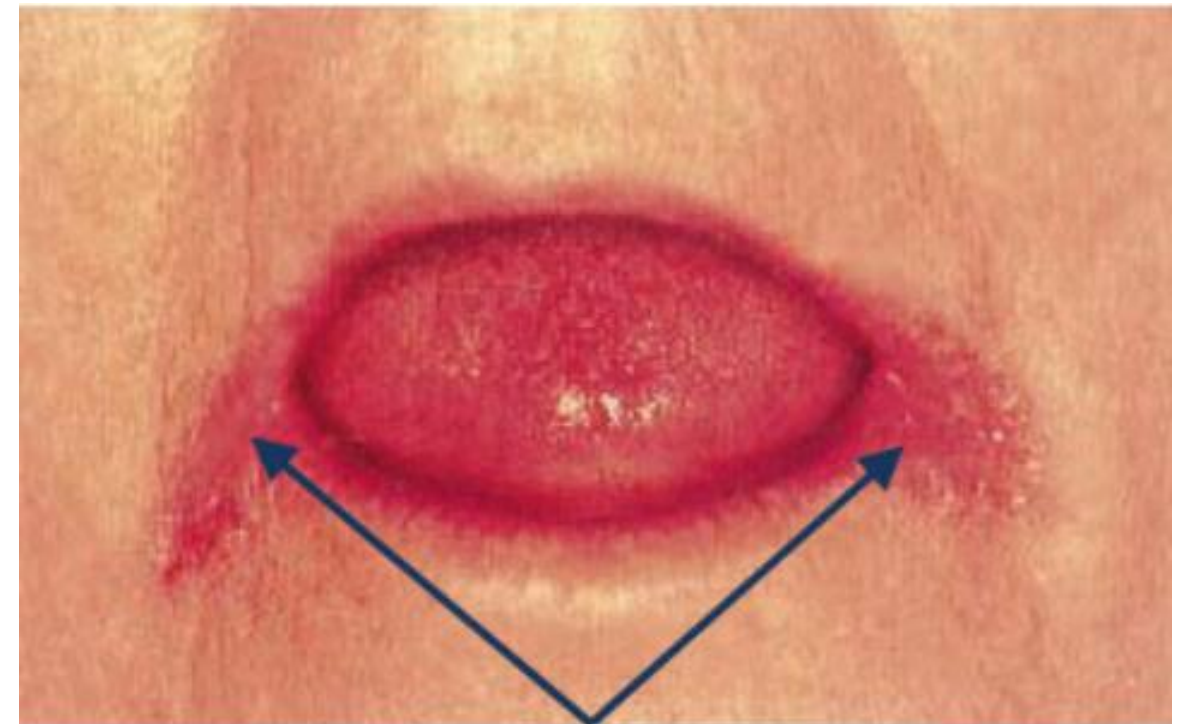


Photo showing bilateral
Angular Cheilitis

Nutrient Deficiencies – Physical Manifestations

Vitamin B2

- sore/reddened tongue, angular stomatitis, gingivitis
- Nasolabial dyssebacia (red, inflamed, oil producing sebaceous glands on sides of nose)



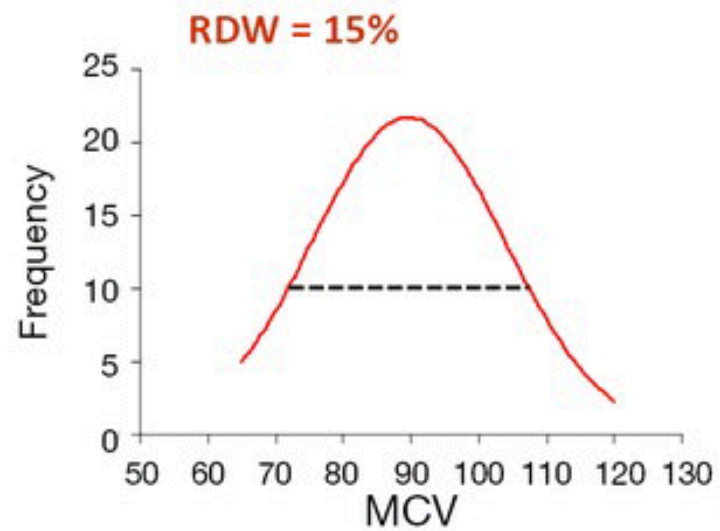
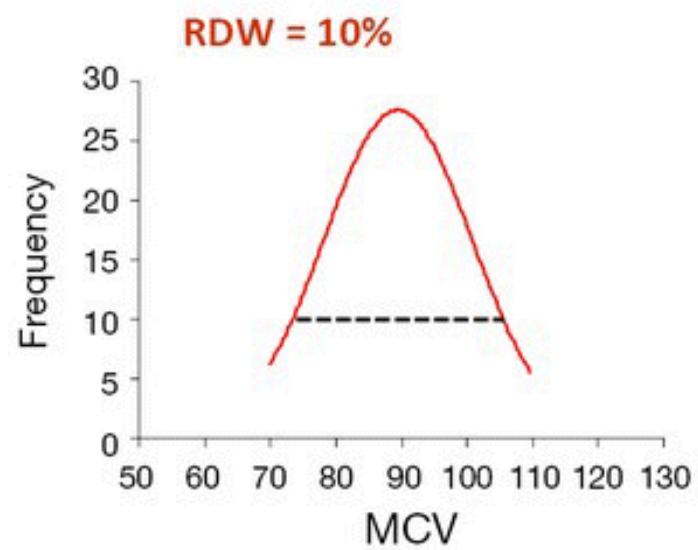
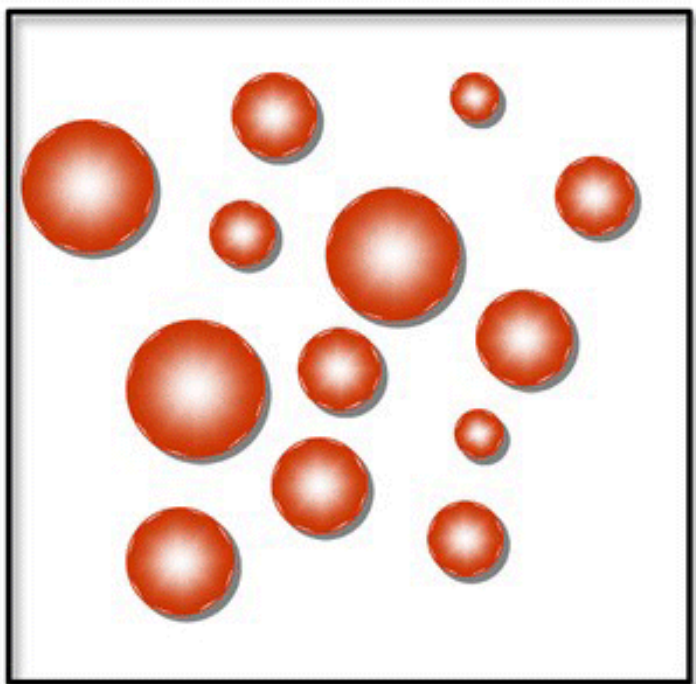
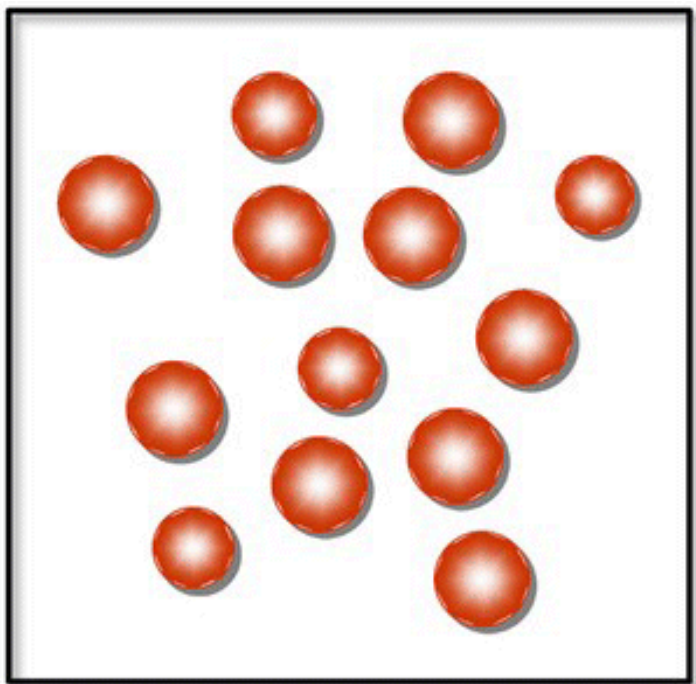
Nutrient Deficiencies – Physical Manifestations

Vitamin B1

- Dementia, ataxia (with poor reflexes), nystagmus
- Tachycardia, intermittent claudication
- Muscle pain, calf tenderness/weakness

Red Cell Distribution Width (RDW)

- * Indication of the variation in red cell size, called anisocytosis.
- * Red blood cells normally have some variation in size, hence having a traditional reference range of 11.5-14.5%
 - * Optimal reference range is <12%
- * The RDW is helpful in identifying an anemia of nutrient deficiency versus other types, such as blood loss, hemolysis, etc
- * Generally speaking, when RDW is normal and anemia is present, it is likely *not* due to nutrient deficiency. If RDW is elevated and anemia is present, it is likely due to nutrient deficiency (eg iron, B12, folate, B6, etc).
- * If no anemia is present, RDW might be used as an inflammatory marker



Anisocytosis

Relation Between Red Blood Cell Distribution Width and Inflammatory Biomarkers in a Large Cohort of Unselected Outpatients

Giuseppe Lippi, MD; Giovanni Targher, MD; Martina Montagnana, MD; Gian Luca Salvagno, MD; Giacomo Zoppini, MD; Gian Cesare Guidi, MD

• **Context.**—A strong independent association has been recently observed between elevated red blood cell distribution width (RDW) and increased incidence of cardiovascular events.

RDW quartiles, there were strong, graded increases of ESR and hsCRP ($P < .001$), both parameters being up to 3-fold higher in the fourth versus the first quartile. Accordingly, the percentages of those with hsCRP greater than 3 mg/L

To assess whether RDW is associated with plasma markers of inflammation since the mechanism(s) underlying this association remain unknown.

...a strong, graded association of RDW with hsCRP and ESR independent of numerous confounding factors. If confirmed in future follow-up studies, this association might provide a rationale to introduce the easy, inexpensive RDW in algorithms for cardiovascular risk prediction.

marker, since it appears to be a stronger predictor than total cholesterol, and it adds prognostic value to the Framingham risk score assessment.^{1,2} The link between hsCRP and atherosclerosis was initially suggested to be that of a biomarker versus a mediator of atherosclerosis. This dog-

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The authors have no relevant financial interest in the products or companies described in this article.

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628 Arch Pathol Lab Med—Vol 133, April 2009

ments are referred to as the mean corpuscular volume (MCV) and the RBC distribution width (RDW), respectively.⁵ Red blood cell distribution width is an automatically measured index of the heterogeneity of the erythrocytes that is calculated by dividing the standard deviation (SD) of RBC volume by MCV and multiplying by 100 to express the result as a percentage.⁶ A high RDW is generally set at greater than 14%, which corresponds to the 95th percentile of RDW for the reference population in the National Health and Nutrition Examination Survey III study.⁶ Elevated RDW indicates a greater difference in size among RBCs, and when accompanied by a low MCV is indicative of iron deficiency, whereas normal RDW with low MCV is indicative of thalassemia.⁵ Very recently, RDW was found to be a strong, independent predictor of morbidity and mortality in 2 large contemporary heart failure populations.⁷ Moreover, Tonelli et al⁸ found that an in-

RDW and Inflammatory Biomarkers—Lippi et al

SCIENTIFIC REPORTS

Red blood cell distribution width (RDW) is the coefficient of variation of red blood cell size, considered to be associated with cardiovascular disease (CVD). This study aimed to comprehensively synthesize previous studies on RDW and CVD outcomes through an overall and dose-response meta-analysis

Twenty-seven articles, consisting of 28 studies and 102,689 participants (mean age 63.9 years, 63,703 males/36,846 females, 2,140 gender-unmentioned subjects) were included in the present meta-analysis.

The pooled HRs are 1.12 (95% CI = 1.09–1.15) for the association of all-cause mortality (ACM) per 1% increase of RDW, 1.12 (95% CI = 1.08–1.17) for major adverse cardiac events (MACEs) per 1% increase of RDW.

For every 1-unit increase of RDW, there is an increased risk of occurrence of ACM and MACEs. This study indicates RDW may be a prognostic indicator for CVD outcomes.

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SCIENTIFIC REPORTS

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An overall and dose-response meta-analysis of red blood cell

distribution width with CVD outcomes

Hou^{1,2,*}, Tao Sun^{1,*}, Cheng Li³

Red blood cell distribution width (RDW) is associated with cardiovascular disease. Previous studies on RDW and CVD outcomes used Embase and Web of Science were conducted up to November 30, 2015. We performed a meta-analysis for calculating pooled hazard ratio (HR) and 95% confidence interval (CI). Twenty-seven articles, consisting of 10,140 males/36,846 females, 2,140 gender-specific studies were included in the meta-analysis. The pooled HRs are 1.12 (95% CI = 1.02–1.23) per 1% increase of RDW. A dose-response curve was constructed ($p_{\text{curve}} < 0.001$). For every 1-unit increase of RDW, the pooled HR = 1.03, 95% CI = 1.02–1.04. This indicates RDW may be a prognostic factor for CVD outcomes.

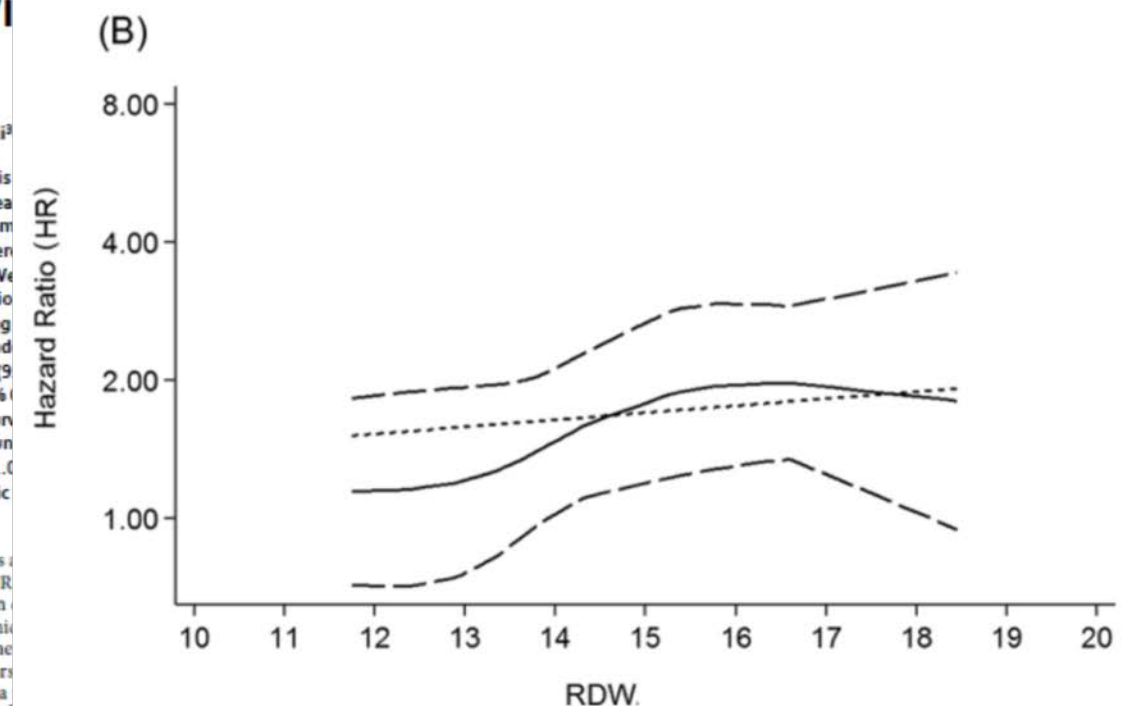
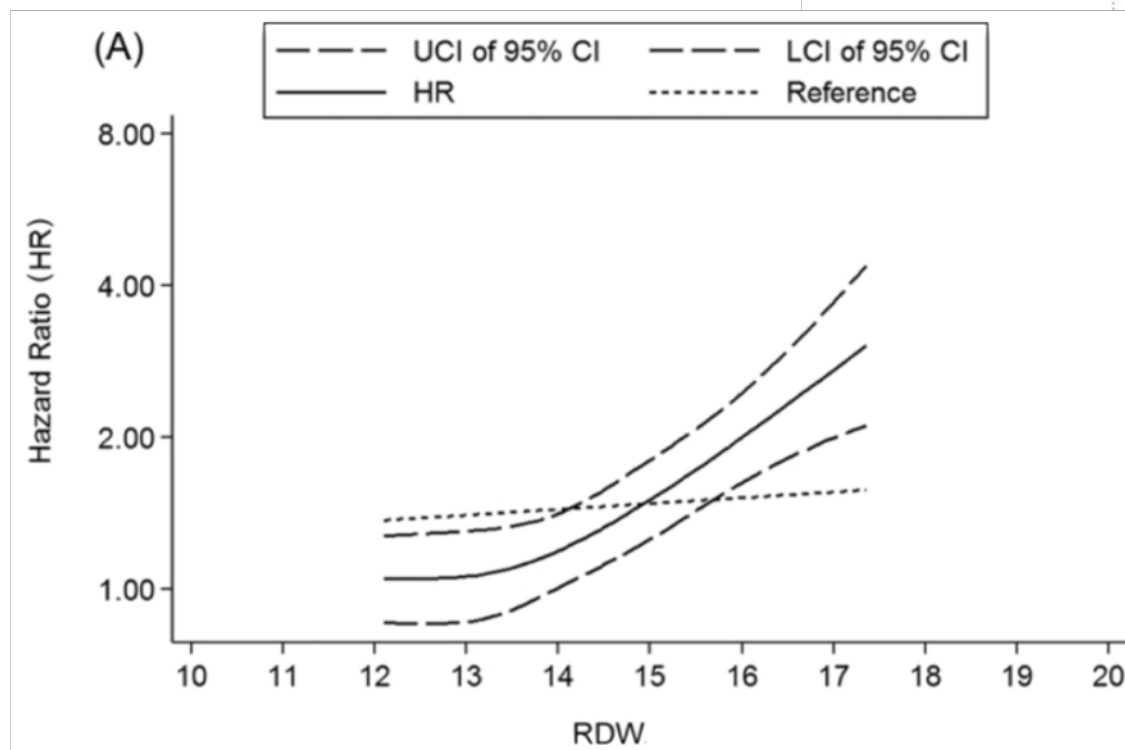
Red blood cell distribution width (RDW) is a measure of the variation of red blood cell size. Red blood cell size (equal to standard deviation of red blood cell size) has been studied for a long time^{1,2}. Recently, the clinical significance of RDW in cardiovascular disease (CVD), autoimmune disease, and other diseases has been reported. RDW is one of significant indicators of CVD. Patients have reported that RDW is a prognostic factor for adverse cardiac events (MACEs) as well as mortality.

MACEs include cardiovascular death, nonfatal myocardial infarction, and nonfatal stroke. However, the results are inconsistent and the underlying mechanism is unclear. We combined the findings of RDW on predicting cardiovascular risk^{3–10}; no dose-response meta-analysis has been performed¹¹. Thus, we conducted the first overall and dose-response meta-analysis to offer new evidence on the association of RDW with CVD outcomes. The prognostic value of RDW for ACM and MACEs were then comprehensively reviewed among CVD (e.g. coronary artery disease, HF, MI) patients.

Results

Search results and study characteristics. As shown in flowchart (Fig. 1), our literature search returned 1,498 publications. After screening the title and abstract of these articles, we kept 126 ones for full-text review. Consequently, this meta-analysis included 27 articles with 28 independent studies and 102,689 participants.

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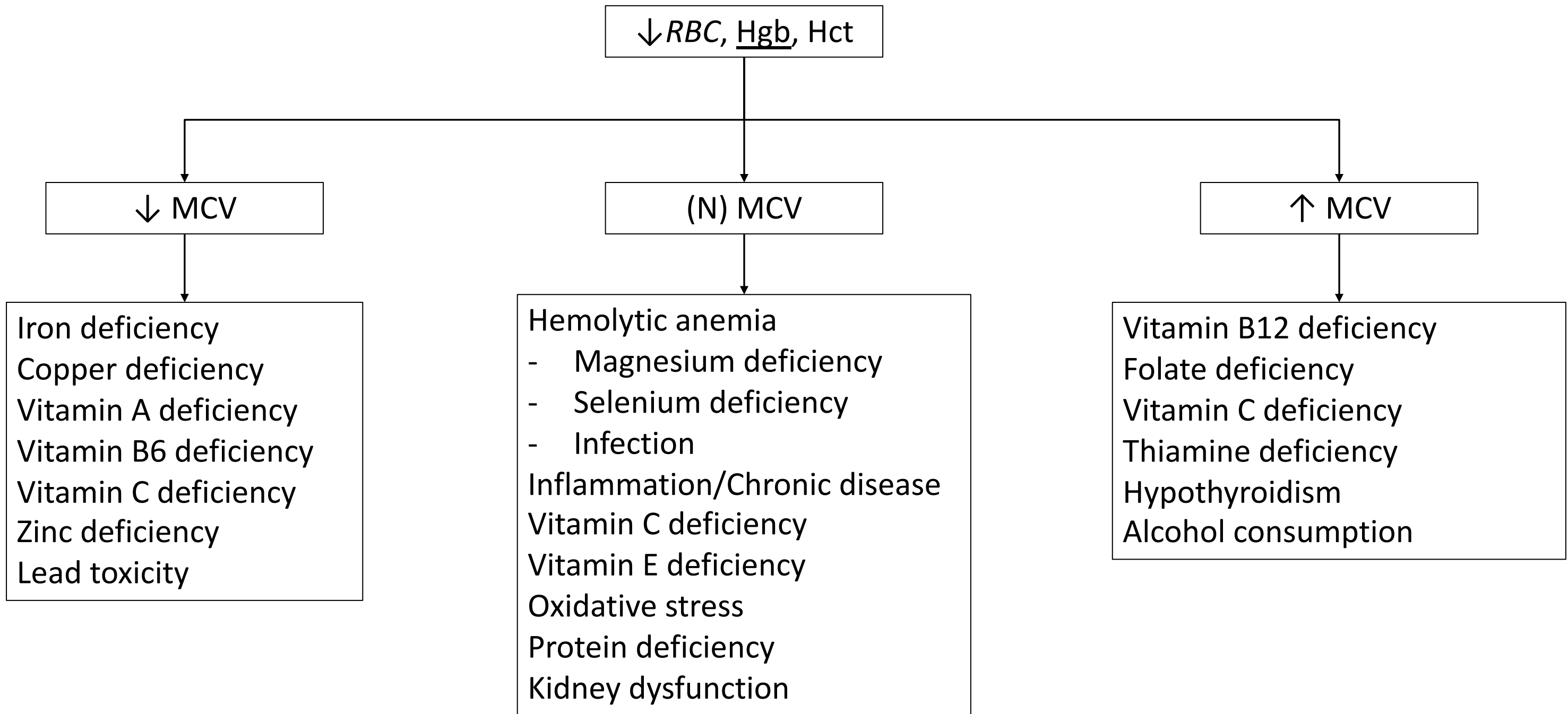
Dose-response relationship between RDW (per 1-unit increase) and CVD outcomes.

Anemia

- * RBC, hemoglobin, hematocrit – if decreased, suggest anemia (inability to optimally deliver oxygen to cells of the body)
- * MCV, MCH, MCHC – suggest the cause or type of anemia
- * RDW – can help indicate whether the anemia is of a nutrient deficiency or not

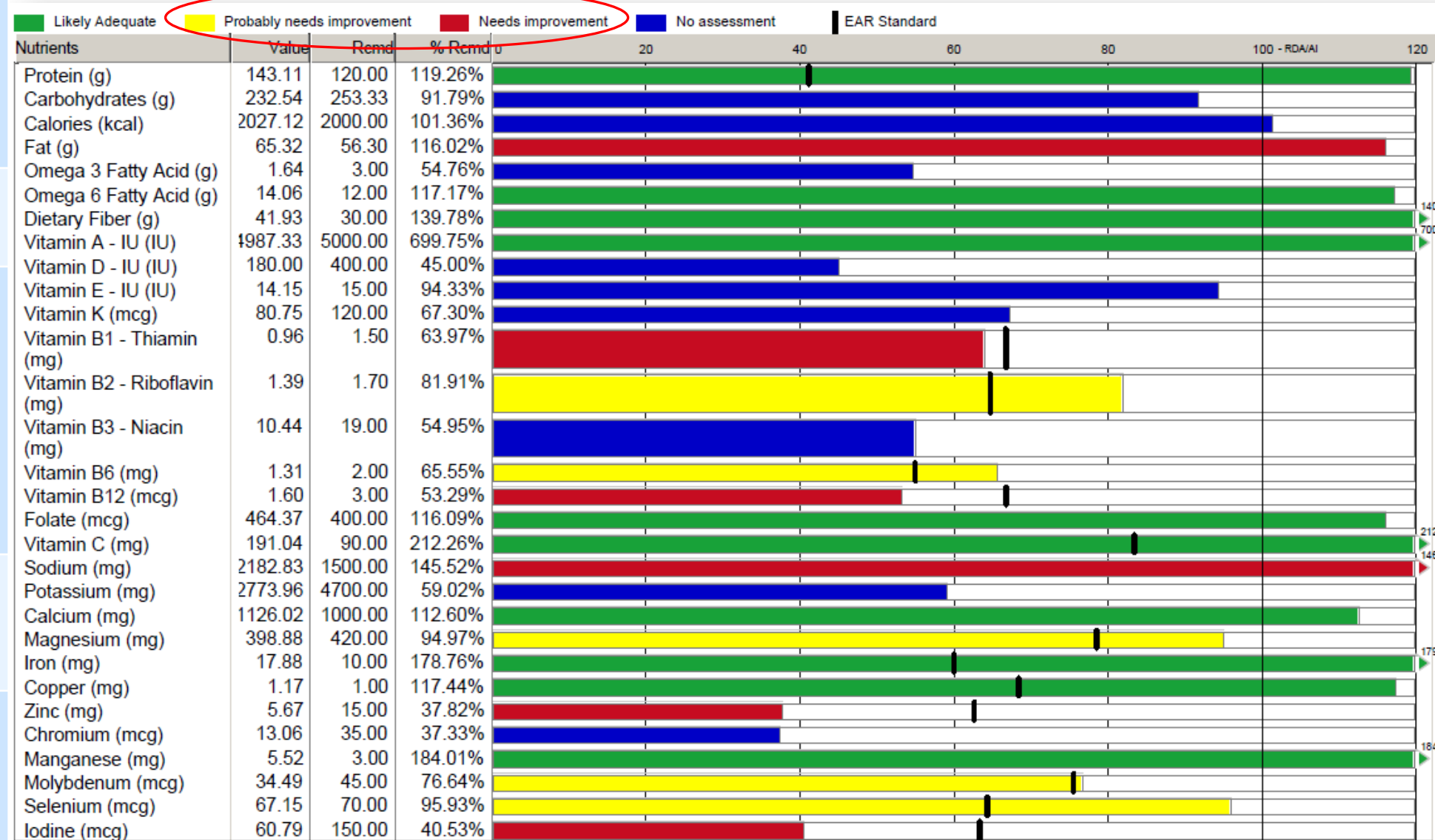
Anemia

1. Production
 - * Nutrient deficiencies, lack of production stimulus
2. Destruction
 - * RBC are being broken down more rapidly than they can be produced
 - * Nutrient deficiency, infection
3. Loss
 - * Red blood cells are being lost via abnormal bleeding



Why Supplement?

Meal	Food	Portion
Breakfast	Hard boiled eggs	2 each
	100% multigrain bread	2 slices
	Natural almond butter	1 Tbsp
	Fresh orange juice	4 oz.
Snack	2% cottage cheese	½ cup
	Fresh Blueberries	1 cup
Lunch	Grilled chicken breast	4oz.
	Romaine lettuce	4 cups
	Fresh red onion, 1/8" thick	4 pieces
	Cucumber slices	½ cup
	Cherry tomatoes	½ cup
	Caesar salad dressing	2 Tbsp
Snack	Dry roasted peanuts	1 oz.
	Medium apple	1 each
	Skim milk	8 oz.
Dinner	Grilled Salmon	6 oz.
	Brown rice	1.5 cup
	Mixed vegetables	2 cups
	Steamed spinach	1 cup



2048 Calories, 45% Carb, 28% fat, 28% Pro

Differentiating Between Destruction and Loss

	Anemia Due to Blood Loss	Anemia of Inflammation	Hemolytic Anemia	Iron Deficiency
RBC	↓	↓	↓	↓
Hgb	↓	↓	↓	↓
Hct	↓	↓	↓	↓
MCV	N	N↓	N↓	↓
MCH	N	N↓	N↓	↓
MCHC	N	N↓	N↓	↓
RDW	N	N	N	↑
Iron	↓		↑	↓
Ferritin	↓	↑	↑	↓
TIBC	↑			↑
Reticulocyte Count	↑	N↓	↑	N↓
Transferrin Receptor		N↓		↑

	Iron Deficiency	Vitamin A Deficiency	Copper Deficiency	Zinc Deficiency	Vitamin B6 Deficiency	Hemolytic Anemia
RBC	↓	↓	↓	↓	↓	↓
Hgb	↓	↓	↓	↓	↓	↓
Hct	↓	↓	↓	↓	↓	↓
MCV	↓	↓	↓	↓	↓	N↓
MCH	↓	↓	↓	↓	↓	N↓
MCHC	↓	↓	↓	↓	↓	N↓
RDW	↑	↑	↑	↑	↑	N
Iron	↓	↑	↓	↑	↑	↑
Ferritin	↓	↑	↓	↑	↑	↑
TIBC	↑	↓	↑	↓	↓	↓
Reticulocyte Count	↓	↓	↓	↓	↓	↑
Transferrin Receptor	↑		↑			
Ceruloplasmin/ Copper			↓			
AST					↓	
ALT					↓	
Neutrophils	N		↓			
Homocysteine			268		↑	
Alkaline Phosphatase				↓		

Combined Iron and B12 Deficiency

- * If both iron and B12 are deficient, the MCV might be normal
 - * Iron deficiency leads to microcytosis (low MCV), B12 deficiency leads to macrocytosis (high MCV)
- * Research suggests the platelet/MCH ratio can help differentiate between the two
 - * In patients with iron deficiency (low ferritin), a platelet/MCH ratio >12.0 was indicative of a possible B12 deficiency as well
 - * Platelets 350, MCH 26: $350/26 = 13.5$

Iron

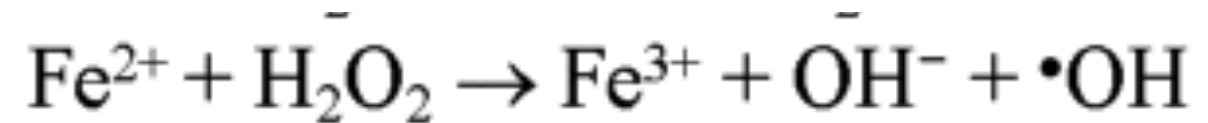
- * Serum iron concentration measures iron bound to transferrin.
- * This value represents approximately one-third of the total iron binding capacity (TIBC) of transferrin protein, which is made largely in the liver.
- * TIBC measures the iron-binding capacity of transferrin.
- * In iron deficiency anemia, TIBC will increase due to an increase in production of transferrin.

Iron

- * Iron by itself is a relatively poor marker of iron status and can fluctuate considerably.
- * Iron has many functions in the body including:
 - * Hemoglobin and myoglobin
 - * Cytochrome enzymes in the Electron Transport Chain (ATP synthesis)
 - * Monooxygenase enzymes (dopamine and serotonin synthesis)
 - * Peroxidases (catalase, myeloperoxidase, thyroperoxidase)
 - * Collagen and carnitine synthesis

Iron

- * Can also create *massive* amounts of oxidative stress via a process called the Fenton reaction



- * Iron absorption is enhanced by sugars (fructose), acids (ascorbic), meat.
- * Iron absorption is inhibited by polyphenols (tannins), oxalic acid (spinach, tea), phytates, other minerals (zinc, calcium)

Iron

Traditional Reference Range

- * 40-155 mcg/dL

Optimal Reference Range

- * 80-100 mcg/dL

Transferrin saturation % = (serum iron X 100)/TIBC

Example:

- * Serum iron = 100
- * TIBC = 300 mcg/dL
- * Transferrin saturation = 33%
 - * The lower the saturation, the more likely iron deficiency exists

Iron - Elevated

Cause	Reason	Additional Inquiry
Hemosiderosis, hemochromatosis	Genetic conditions clients are likely already aware of. Iron overload.	Client history.
Hemolytic anemia	If RBCs break-down, iron will be released and can increase in the serum.	Evaluate CBC markers.
Liver damage	Liver and spleen are major sources of iron storage. Thus if there is active liver inflammation iron levels may increase.	Evaluate liver markers.
Vitamin B6 deficiency	If B6 is not available for heme synthesis, iron will not be used and can increase.	Evaluate AST, ALT.

Iron - Decreased

Cause	Reason	Additional Inquiry
Poor intake		Diet journal.
Poor absorption		Diet journal.
Chronic blood loss	Heavy menstruation, GI bleed	Client history. Reticulocytes. CBC.
Chronic disease and/or chronic infection	Iron either becomes depleted or sequestered.	Client history.
Progesterone birth control pills	Association	Client history.

Transferrin/TIBC - Elevated

Cause	Reason	Additional Inquiry
Iron deficiency	Increased production.	Evaluate ferritin.
Pregnancy		
Elevated estrogen	Estrogen tends to increase plasma protein production, including transferrin.	Client history for exogenous use. Evaluate T3 Uptake.

Transferrin/TIBC - Decreased

Cause	Reason	Additional Inquiry
Anemia of Chronic Disease	Transferrin is a negative acute phase reactant, meaning it will tend to decrease during chronic inflammation and/or infection	Client history.
Chronic infection	Same as above	Client history. Evaluate WBCs.
Liver dysfunction	Poor production.	Evaluate liver markers.

Ferritin

- * Ferritin is considered to be the body's stored form of iron.
- * It is the ferrous form of iron (Fe^{2+}) complexed with a protein, apoferritin, storing it in the ferric state (Fe^{3+}).
- * Most reliable indicator of total-body iron status.
- * Ferritin is an *acute phase reactant*, in that it will increase during inflammation or infection.

Ferritin

Traditional Reference Range

- * Men: 18-270 ng/mL or 18-270 ug/L
- * Women: 18-160 ng/mL or 18-160 ug/L

Optimal Reference Range

- * 50-150 ng/mL (Iron Disorders Institute's Scientific & Medical Advisory Board)
- * Women: 50-100 ng/mL?*
- * Men: >100 ng/mL?*

Is serum ferritin within the reference range a risk predictor of cardiovascular disease? A population-based, long-term study comprising 2874 subjects

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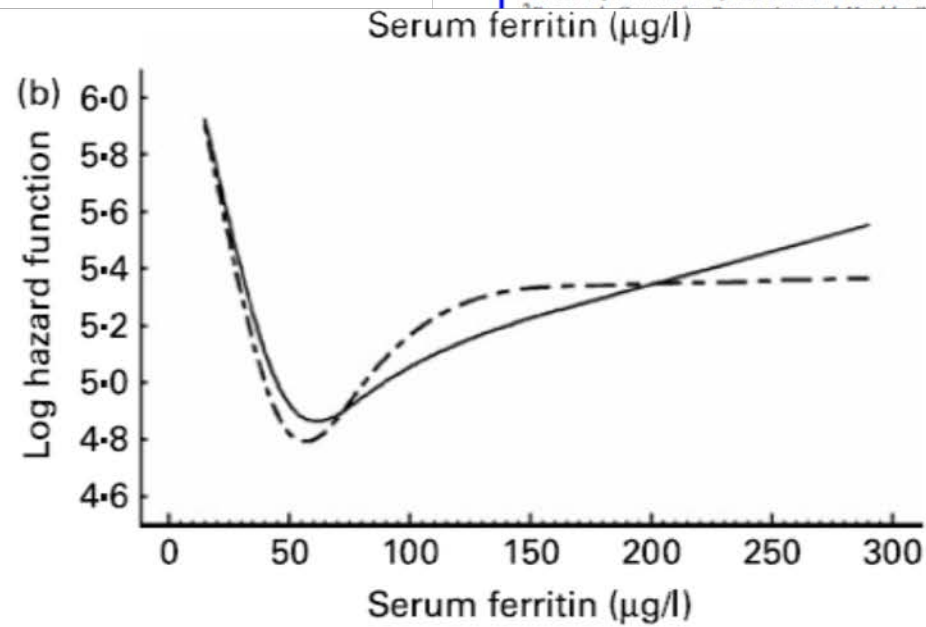


Fig. 1. Predicated log hazard function for CVD as a function of serum ferritin in men (a) and women (b). Results of Cox proportional hazard regression models with restricted cubic splines adjusted for age (—) and fully adjusted (---). The full model was adjusted for age, smoking, alcohol consumption, diabetes, hypertension, serum cholesterol levels and systolic blood pressure.

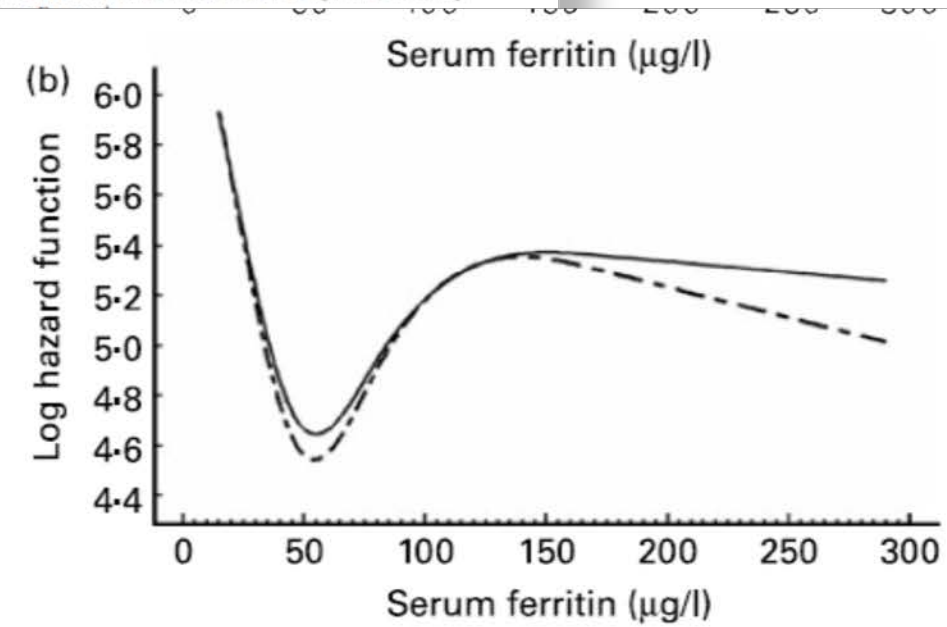


Fig. 2. Predicated log hazard function for IHD as a function of serum ferritin in men (a) and women (b). Results of Cox proportional hazard regression models with restricted cubic splines adjusted for age (—) and fully adjusted (---). The full model was adjusted for age, smoking, alcohol consumption, diabetes, hypertension, serum cholesterol levels and systolic blood pressure.

that ferritin represents a strong predictor of myocardial infarction (relative hazard per unit 1.002 (95% CI 1.001, 1.003). Furthermore, this study reported a 2.2-fold increased risk of myocardial infarction among men with serum ferritin levels $> 200 \mu\text{g/l}^{(8)}$ compared with men with lower levels. Against the original Fe hypothesis, which did not precisely define the association between ferritin and IHD, this paper

studies mentioned^(8,9,15,17) considered the full serum ferritin range. However, the effects of pathological serum ferritin levels are well investigated^(20–23). Therefore, the objective of the present study was to investigate the associations between serum ferritin levels within the reference range and the risks of non-fatal and fatal CVD as well as IHD in 2874 Danish subjects who were followed for 10 years.

Abbreviations: DAN-MONICA, Monitoring of Trends and Determinants in Cardiovascular Disease, Danish part; HR, hazard ratio.

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Serious Gastrointestinal Pathology Found in Patients With Serum Ferritin Values ≤ 50 ng/ml

TABLE 2
Serious Gastrointestinal Pathology Found in Patients With Serum Ferritin Values ≤ 50 ng/ml

Upper gastrointestinal tract	53 (40% of total 132 patients)
Cancer	2
Esophagitis (grades III, IV)	15
Ulcers and erosions of the stomach and/or duodenum	31
Multiple angiodysplasia	5
Lower gastrointestinal tract	29 (23% of total of 125 patients)
Cancer	13
Adenoma > 1 cm	6
Colitis	4
Erosions/ulcers	2
Multiple angiodysplasia	4

Endoscopic examination of the gastrointestinal tract in patients with unexplained iron deficiency anemia shows that approximately half have serious gastrointestinal pathology, including cancer, large polyp, and peptic ulcer disease. In most cases, an evaluation of the gastrointestinal tract is indicated, usually with colonoscopy and/or esophagogastroduodenoscopy (EGD) (1-6). Although the definitive di-

agnostic study data were abstracted from the medical records, endoscopy database, and/or interview of the patient and the primary care physician for eligible patients who were already undergoing gastrointestinal evaluation. The remaining subjects were contacted after obtaining permission from their primary care physicians and asked to undergo colonoscopy and/or EGD at their earliest convenience. We did not deliberately select patients for evaluation based on any particular laboratory or clinical features, but rather enrolled the subjects in the order they were identified.

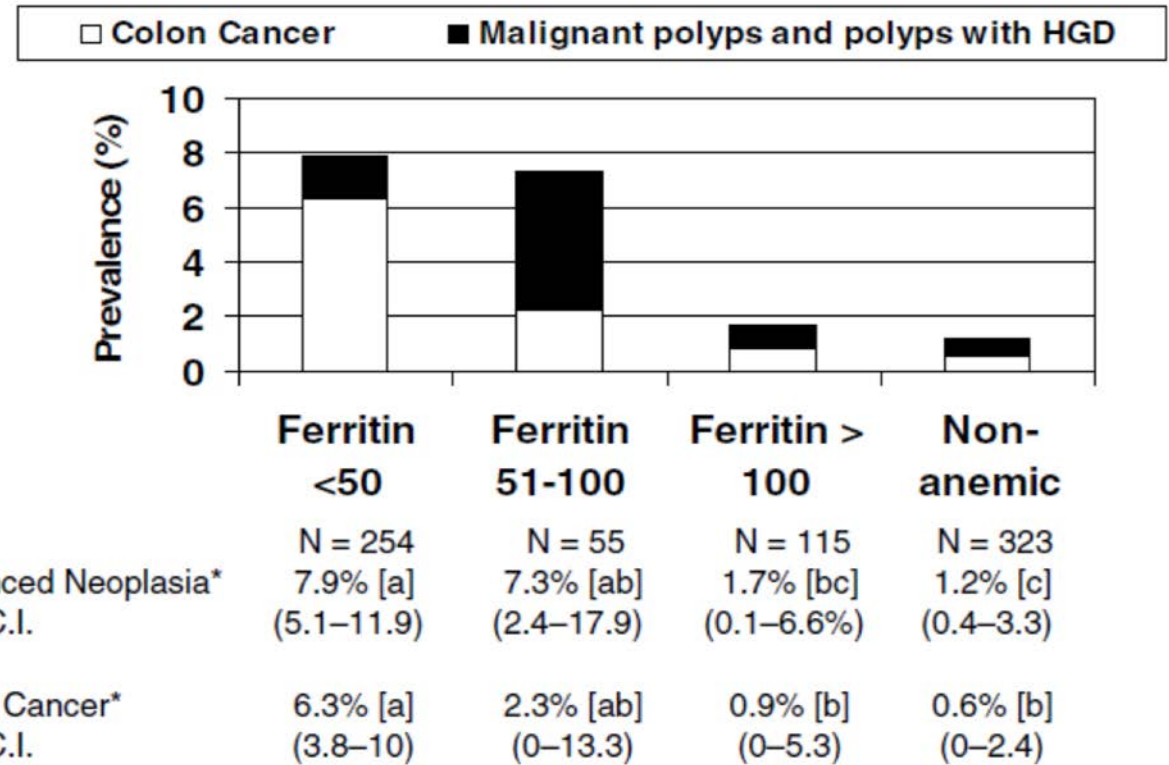
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Should Patients With Anemia and Low Normal or Normal Serum Ferritin Undergo Colonoscopy?

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anemia is a well-recognized sign of colon cancer (2, 3). There is broad consensus among physicians that persons with unexplained iron deficiency anemia be considered for a colonoscopy to exclude colonic neoplasm. The diagnosis of iron deficiency anemia should be firmly established before patients are subjected to costly and invasive procedures. Serum ferritin has been proven to be the most accurate non-invasive test for the diagnosis of iron deficiency anemia, and is often used to select patients for colonoscopy (4, 5). While prospective studies have shown that patients with serum ferritin <50 ng/mL have a high prevalence of colonic neoplasia and should undergo prompt colonoscopy, the prevalence of colonic neoplasia in anemic patients with ferritin level >50 ng/mL is not known (6, 7). There are no established guide-

deficiency is however unlikely, even in the presence of concomitant medical illnesses, if ferritin levels are above 100 ng/mL (10).

Several studies have reported on the yield of colonoscopy in patients with iron-deficiency anemia (2, 3, 6, 7, 11–14). Most are limited to patients with ferritin <50 ng/mL. The determination of whether patients with anemia and ferritin levels of >50 ng/mL are at greater risk for colonic neoplasm is difficult due to the absence of comparison to a nonanemic control group. Prior to the use of screening colonoscopy, it was difficult to accurately ascertain the prevalence of colonic neoplasia in the normal population.

Should patients with anemia and serum ferritin >50 ng/mL undergo colonoscopy? Should patients with ferritin

Ferritin

Traditional Reference Range

- * Men: 18-270 ng/mL or 18-270 ug/L
- * Women: 18-160 ng/mL or 18-160 ug/L

Optimal Reference Range

- * 50-150 ng/mL (Iron Disorders Institute's Scientific & Medical Advisory Board)
- * Women: 50-100 ng/mL?*
- * Men: >100 ng/mL?*

Ferritin - Elevated

Cause	Reason	Additional Inquiry
Hemosiderosis, hemochromatosis	Genetic conditions clients are likely already aware of. Iron overload.	Client history.
Inflammatory diseases	Acute phase reactant.	Evaluate other inflammation markers.
Liver damage	Liver and spleen are major sources of iron storage. Thus if there is active liver inflammation iron levels may increase.	Evaluate liver markers.
Hemolytic anemia, sideroblastic anemia	Iron is being lost or unused from RBCs, thus leading to excess iron.	Evaluate CBC.

Ferritin - Decreased

Cause	Reason	Additional Inquiry
Poor intake		Diet journal.
Poor absorption		Diet journal.
Chronic blood loss	Heavy menstruation, GI bleed	Client history.
Chronic disease and/or chronic infection	Iron either becomes depleted or sequestered.	Client history.
Progesterone birth control pills		Client history.

Reticulocyte Count

- * Reticulocytes are early red blood cells that are formed in the bone marrow, enter the blood stream and, after 1-2 days become fully matured red blood cells.
- * They do not contain a nucleus, but rather have nucleic acid remnants, which are not found in fully formed red blood cells.
- * Reticulocytes tend to be elevated in blood loss and/or hemolytic anemia but normal, or even low, in anemia of nutrient deficiency
 - * Poor RBC production due to low nutrient deficiency – they cannot make more
 - * But respond rapidly to nutrient supplementation by increasing production
 - * Thus reticulocytes can be helpful in seeing if nutrient supplementation is working or not

Reticulocyte count - Increased

Cause	Reason	Additional Inquiry
Hemolytic anemia	Breakdown of red blood cells leads to low oxygen state, which is sensed by the kidneys and increases EPO production	
Blood loss	Same	Client history.

Reticulocyte count - Decreased

Cause	Reason	Additional Inquiry
Untreated nutrient deficiency anemia (iron, B12)	Nutrient deficiency leads to poor RBC production, despite attempts by the body to increase production	Evaluate nutrient-related markers.
Anemia of chronic disease	Inflammation suppresses the ability to make more red blood cells.	Evaluate other inflammatory markers.
Alcoholism	Causes nutrient deficiency	History

Reticulocyte Production Index

- * Reticulocyte “count” is a percentage of RBC, not an absolute count
- * Thus, in anemia, low RBCs may create an increased reticulocyte “count”

Reticulocyte Production Index

Reticulocyte count =

- * number of reticulocytes counted/number of erythrocytes counted

Corrected Reticulocyte Count =

- * Reticulocyte % X Hct/Normal hematocrit (usually 45)

Reticulocyte Production Index =

- * Corrected Reticulocyte Count X Correction Factor

Correction of Reticulocyte Count	
Hematocrit (%)	Reticulocyte Life Span (RLS)
36-45	1.0
26-35	1.5
16-25	2.0
<15	2.5
Adapted from Harrisons Principles of Internal Medicine, 18 th edition.	

Reticulocyte Production Index

Reticulocyte count = 0.8%

- * number of reticulocytes counted/number of erythrocytes counted

Corrected Reticulocyte Count =

- * Reticulocyte % X Hct/Normal hematocrit
- * $0.8 \times 38/45 = 0.6755$ or 0.7%

Reticulocyte Production Index =

- * Corrected Reticulocyte Count X Correction Factor
- * $0.7 \times 1.0 = 0.7$

Correction of Reticulocyte Count	
Hematocrit (%)	Reticulocyte Life Span (RLS)
36-45	1.0
26-35	1.5
16-25	2.0
<15	2.5
Adapted from <i>Harrisons Principles of Internal Medicine</i> , 18 th edition.	

Reticulocyte Production Index

RPI <2 (with anemia)

- * Loss of red blood cells
- * Likely due to production issue (i.e. bone marrow)

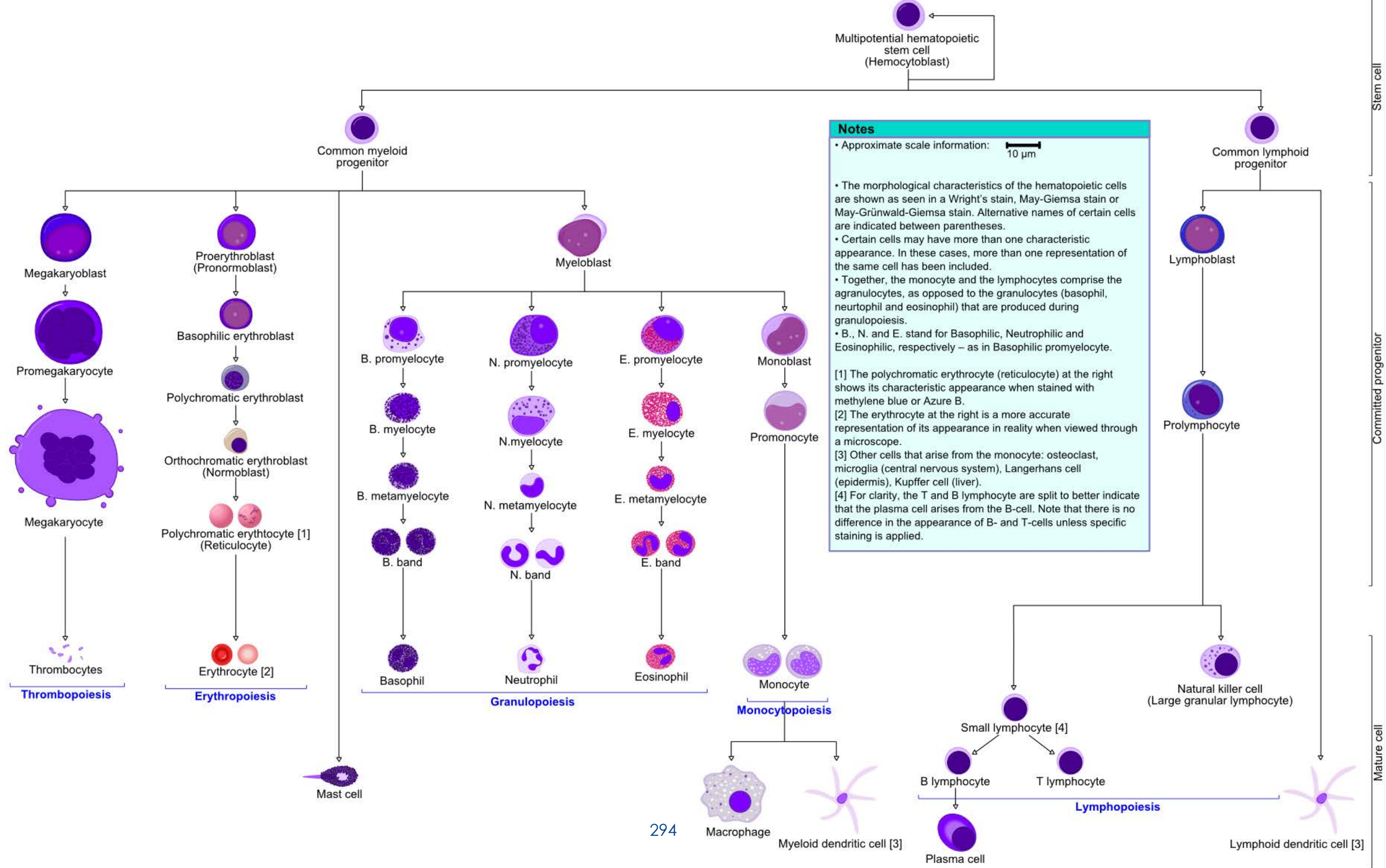
RPI >3 (with anemia)

- * Loss of red blood cells
- * Due to destruction or loss (i.e. bleeding)

Correction of Reticulocyte Count	
Hematocrit (%)	Reticulocyte Life Span (RLS)
36-45	1.0
26-35	1.5
16-25	2.0
<15	2.5
Adapted from <i>Harrisons Principles of Internal Medicine</i> , 18 th edition.	

White Blood Cell Count

- * Refers to total amount of white blood cells
- * The CBC with differential includes the five major types of white blood cells:
 - * Neutrophils, lymphocytes, monocytes, eosinophils, basophils
- * The WBC count is of limited value without the differential
- * In general, an elevated WBC indicates an infection of an acute nature, and a decreased WBC count indicates an infection of a more chronic nature.
 - * Decreased WBCs are often seen in autoimmune conditions and pernicious anemia as well.
- * Interestingly, high-normal white blood cell counts, along with neutrophils, can indicate possible endothelial dysfunction and peripheral artery disease



White Blood Cell Count

Interfering factors include:

- Stress: epinephrine response can increase white blood cells
- Time of day: lower in the morning, higher in the evening
- Other: age, gender, exercise, pregnancy, pain, temperature and altitude can all affect results
- Early infection/inflammation: Migration out of blood may temporarily lower serum numbers before compensation takes place

White Blood Cell Count

Traditional Reference Range:

* $3.4-10.8 \times 10^3/\mu\text{L}$

Leukocyte Count as a Predictor of Cardiovascular Events and Mortality in Postmenopausal Women

The Women's Health Initiative Observational Study

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Background: Increasing evidence supports a role for inflammation in the atherosclerotic process. The role of the leukocyte count as an independent predictor of risk of a first cardiovascular disease (CVD) event remains uncer-

L), women in the fourth quartile ($6.7\text{--}15.0 \times 10^9$ cells/L) had over a 2-fold elevated risk for CHD death (hazard ratio, 2.36; 95% confidence interval, 1.51–3.68), after multivariable adjustment for age, race, diabetes, hyperten-

The WBC count, a stable, well-standardized, widely available and inexpensive measure of systemic inflammation, is an independent predictor of CVD events and all-cause mortality in postmenopausal women. A WBC count greater than 6.7×10^9 cells/L may identify high-risk individuals who are not currently identified by traditional CVD risk factors. (Approximately 50% increase in risk.)

deaths, 701 nonfatal myocardial infarctions, 738 strokes, and 1919 deaths from all causes. Compared with women with WBC counts in the first quartile ($2.5\text{--}4.7 \times 10^9$ cells/

rently identified by traditional CVD risk factors.

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Author Affiliations are listed at the end of this article. A complete listing of the Women's Health Initiative Research Group is given in a box at the end of this article.
Financial Disclosure: Dr Assaf is an employee of Pfizer Inc.

INCREASING EVIDENCE SUPPORTS A role for inflammation in the atherosclerotic process.^{1,2} Initiation, growth, and complications of atherosclerotic plaques are each judged to be an inflammatory response to vascular injury,^{3,4} and inflammatory markers and cytokines originating in the heart, vessel walls, macrophages, adipose tissue, and liver have been associated with the risk of coronary events.⁵ In light of the multitude of pathobiological factors involved in inflammation, a large number of targets for measurement have been proposed to identify and monitor the inflammatory process in patients with, or at risk for, coronary heart disease (CHD). These include proinflammatory factors such as oxidized low-density lipoproteins, pro-

inflammatory cytokines (eg, interleukin 1 and tumor necrosis factor- α), adhesion molecules (eg, intercellular adhesion molecule 1 and selectins), inflammatory stimuli with hepatic effects (eg, interleukin 6), or

*For editorial comment
see page 487*

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the products of the hepatic stimulation, such as serum amyloid A, C-reactive protein (CRP), and other acute-phase reactants.⁶ In addition, indicators of cellular responses to inflammation, such as elevated white blood cell (WBC) count, have also been considered.⁶

Leukocyte Count as a Predictor of Cardiovascular Events and Mortality in Postmenopausal Women

Table 1. Baseline Characteristics of WHI-OS Participants Free of CVD at Baseline, by WBC Count Quartile*

Characteristic	Quartile of WBC Count, $\times 10^9$ cells/L†				Total (N = 72 242)
	Q1, 2.50-4.70 (n = 18 356)	Q2, 4.71-5.60 (n = 18 557)	Q3, 5.61-6.70 (n = 18 133)	Q4, 6.71-15.00 (n = 17 196)	

first cardiovascular event. Our objective was to determine whether the baseline WBC count was associated with CVD events and mortality in postmenopausal women free of CVD at baseline.

Methods: In this prospective cohort study, 161 808 postmenopausal women were enrolled in the Women's Health Initiative Observational Study. Baseline characteristics were assessed at enrollment. Incident CVD events were defined as CHD death, nonfatal MI, stroke, or total CVD.

Results: At baseline, the mean age was 63 ± 7.3 years, 61% had hypertension, and the mean WBC count was 6.1×10^9 cells/L. Over 6 years of follow-up, there were 701 CHD deaths, 1919 nonfatal MIs, 1919 strokes, and 1919 total CVD events.

Author Affiliation: From the Department of Medicine, University of California, San Francisco, CA (Dr. Manson); and the Department of Medicine, University of California, San Francisco, CA (Dr. Manson).
Financial Disclosure: Dr. Manson is an employee of the National Institutes of Health.

Table 2. Association Between WBC Quartile and Incident Cardiovascular Events in WHI-OS Participants Free of Cancer and CVD at Baseline*

WBC Count Quartile†	CVD Events, No. (%)‡	Age, Race/Ethnicity Adjusted HR (95% CI)	Multivariate Adjusted HR§ (95% CI)
CHD death			
Q1	28 (0.2)	1.00 (Referent)	1.00 (Referent)
Q2	24 (0.1)	0.80 (0.46-1.38)	0.73 (0.42-1.26)
Q3	44 (0.3)	1.46 (0.91-2.35)	1.24 (0.77-2.01)
Q4	91 (0.6)	3.15 (2.06-4.83)	2.36 (1.51-3.68)
Total	187 (0.3)		
Nonfatal MI			
Q1	118 (0.7)	1.00 (Referent)	1.00 (Referent)
Q2	140 (0.8)	1.09 (0.85-1.39)	0.98 (0.76-1.25)
Q3	193 (1.2)	1.50 (1.19-1.89)	1.23 (0.97-1.55)
Q4	250 (1.6)	2.05 (1.64-2.55)	1.41 (1.12-1.78)
Total	701 (1.1)		
Stroke			
Q1	137 (0.8)	1.00 (Referent)	1.00 (Referent)
Q2	167 (1.0)	1.12 (0.89-1.41)	1.04 (0.83-1.30)
Q3	168 (1.0)	1.12 (0.89-1.40)	0.97 (0.77-1.23)
Q4	266 (1.7)	1.87 (1.52-2.30)	1.46 (1.17-1.81)
Total	738 (1.1)		
Total CVD			
Q1	265 (1.6)	1.00 (Referent)	1.00 (Referent)
Q2	319 (1.9)	1.11 (0.94-1.31)	1.01 (0.86-1.19)
Q3	381 (2.3)	1.32 (1.13-1.54)	1.12 (0.95-1.31)
Q4	545 (3.5)	1.99 (1.72-2.31)	1.47 (1.26-1.72)
Total	1510 (2.3)		
Total mortality			
Q1	351 (2.1)	1.00 (Referent)	1.00 (Referent)
Q2	407 (2.4)	1.08 (0.94-1.25)	1.00 (0.87-1.16)
Q3	444 (2.7)	1.12 (0.98-1.28)	1.03 (0.90-1.18)
Q4	611 (3.5)	1.61 (1.42-1.83)	1.23 (1.07-1.41)
Total	1813 (2.7)		

CHD, coronary heart disease; MI, myocardial infarction; CVD, cardiovascular disease; HR, hazard ratio; CI, confidence interval; §, adjusted for age, race, ethnicity, body mass index, current smoking status, and hormone therapy use; ||, adjusted for all the above factors plus baseline WBC count.

stable, well-tolerated, and easy to use, and the WBC count was a significant predictor of incident CVD events in postmenopausal women with a WBC count $\geq 6.7 \times 10^9$ cells/L.

g, interleukin 1 receptor- α , adhesion molecules, and inflammatory stimuli (interleukin 6), or

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ic stimulation, C-reactive protein, and proinflammatory phase reactants of cellular response, such as elevated WBC count, have also



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Vascular Endothelial Function is Related to White Blood Cell Count and Myeloperoxidase Among Healthy Middle-Aged and Older Adults

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Abstract

The peak forearm blood flow (FBF) response to intra-brachial artery infusion of acetylcholine was inversely related to WBC count ($r=-0.38$, $P=0.004$) and was 34% smaller in subjects with higher vs. lower WBC count ($>$ vs. $<$ median of 5.0×10^9 cells/L, $P=0.001$). EDD was related ($P<0.05$) to **neutrophil**, eosinophil and monocyte, but not lymphocyte or basophil counts.

is related to higher neutrophil, eosinophil and monocyte-based WBC count mediated by reduced responsiveness to NO and increased myeloperoxidase-associated reductions in tetrahydrobiopterin and NO bioavailability.

Keywords

endothelium-dependent dilation; aging; nitric oxide; tetrahydrobiopterin

Because age is the major risk factor for cardiovascular diseases (CVD), middle-aged and older adults are at elevated risk for CVD in the absence of other conventional risk factors.¹ Much of this increased risk is related to vascular endothelial dysfunction, a key feature of which is an impaired ability of peripheral arteries to dilate in response to a pharmacological or flow-induced stimulus.^{1, 2} Endothelial dysfunction, characterized by impaired endothelium-dependent dilation (EDD), is a predictor of future CVD-related events in older adults without clinical disease at baseline.^{3, 4}

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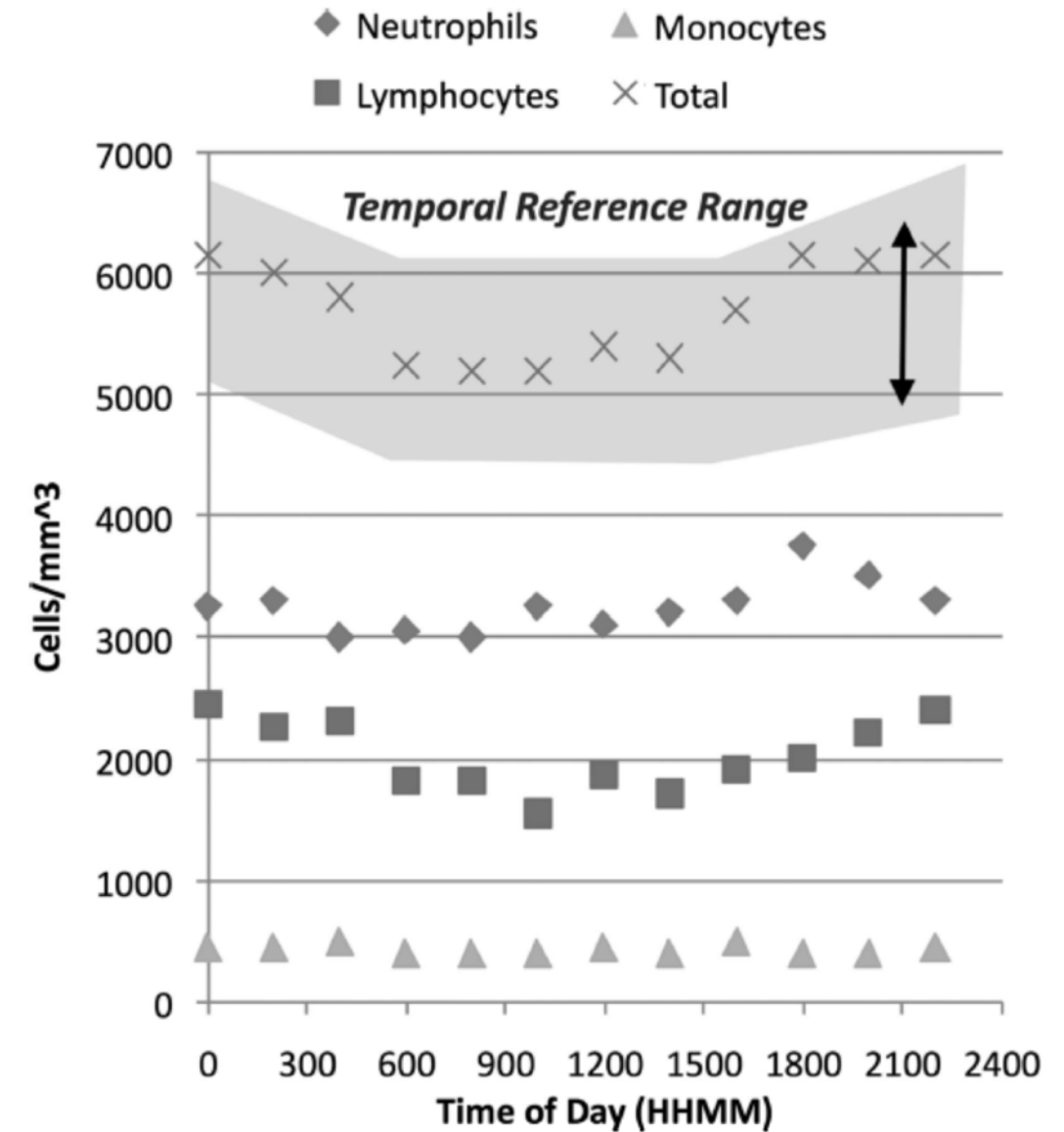
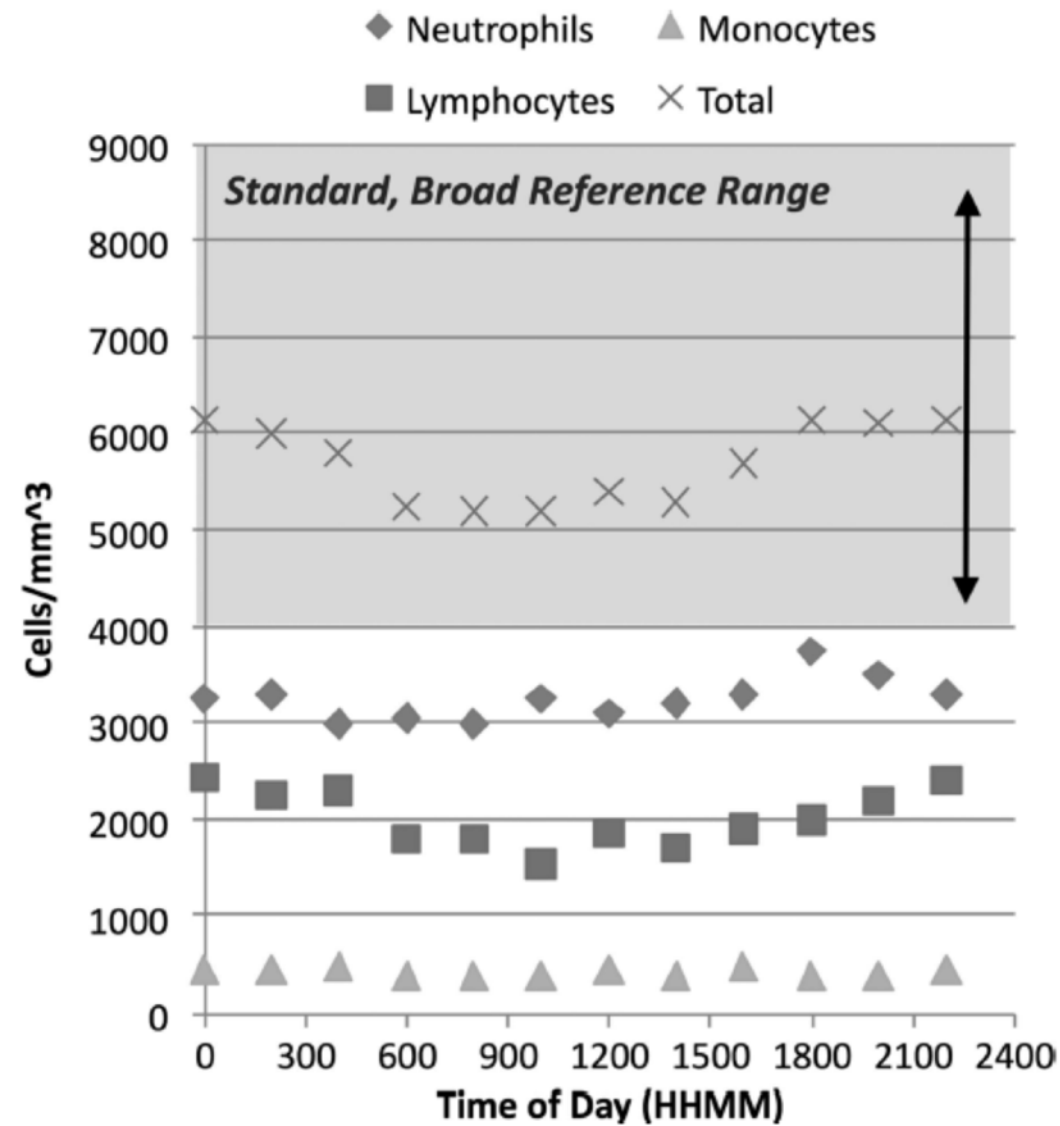
Conflicts of Interest: None

EDD Related to Neutrophils

- * ↑ Neutrophils → ↑ myeloperoxidase → ↓ oxidized tetrahydrobiopterin → ↓ NO production
 - * Myeloperoxidase also consumes NO
- * Result = increased endothelial dysfunction

Complete blood counts with differential: more accurate reference ranges based on circadian leukocyte

We recognise that in some cases, cell counts are likely to be so far outside the reference range that little would be gained by having a more precise, temporally adjusted, reference range, for example, septicaemia or late-stage leukaemia.⁶ Nonetheless, there



that compare a patient's results collected at the same time (Figure 2). This will allow clinicians to detect abnormalities sooner, for earlier diagnosis of pathologies. In clinical protocols, early abnormalities are often missed because the normal ranges are homogenised over a 24-hour period. The reference ranges at the National Clinical Center,⁵ in table 1, are generally quite wide. For example, the range of neutrophils in adult men: one man's count can be three times another (5.38 K/ μ L vs 1.78 K/ μ L), more extreme among the women who have a 900% range of neutrophils (which have an 'normal' range). On the other hand, lymphocytes, which are not trapped in circulation, vary by only 10% and 33% in women.

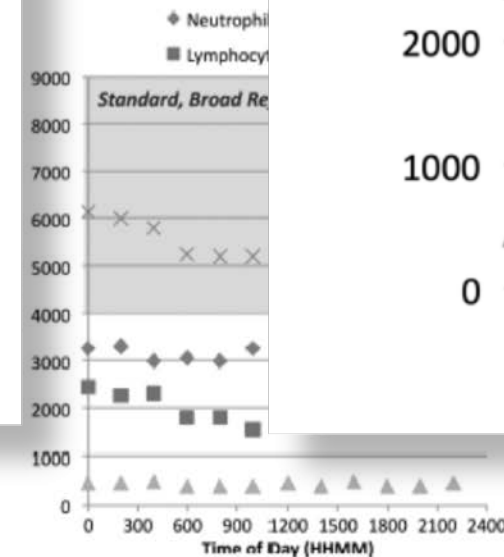


Figure 1 Single metrics reference ranges are unnecessarily inflated because they must include the highs and lows of normal circadian fluctuation in WBCs.

programming them to calculate more precise

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White blood cell count in young adulthood and coronary artery calcification in early middle age: coronary artery risk development in young adults (CARDIA) study

Lifang Hou · Donald M. Lloyd-Jones · Hongyan Ning · Mark D. Huffman · Myriam Fornage · Ka He · Xiao Zhang · David R. Jacobs · David C. Goff · Steve Sidney · Jeffrey J. Carr · Kiang Liu

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We demonstrated that total WBC counts in young adults is associated prospectively with subclinical atherosclerosis measured by CAC 20 years later into middle age, suggesting total WBC and eosinophil counts may be a marker of early stage atherosclerosis.

566 subjects had CAC scores >0 at year (Y) 15 and Y20, respectively. Baseline total WBC counts were cross-sectionally associated with SBP, BMI, and smoking, or HDL-cholesterol ($p \leq 0.01$) at Y0, and prospectively associated

Electronic supplementary material The online version of this article (doi:10.1007/s10654-013-9842-7) contains supplementary material, which is available to authorized users.

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ted for. Our results suggest the possible early involvement of WBC, particularly eosinophils, in the early stages of atherosclerosis.

Keywords White blood cell count · Coronary artery calcification · Atherosclerosis

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White blood cell count in young adulthood and coronary artery calcification in early middle age: coronary artery risk development in young adults (CARDIA) study

Baseline characteristic	Tertiles of total WBC counts, 10 ⁹ /L			<i>p</i> value*
	<5.1	5.1–6.5	>6.5	
Age, years	25.2 ± 3.6	25.2 ± 3.6	25.1 ± 3.6	0.55
Race, Black (%)	51.5	41.2	41.4	<0.01
Sex, Male (%)	48.8	44.6	35.8	<0.01
Education, years	14.1 ± 2.27	14.1 ± 2.2	14.1 ± 2.6	0.61
Systolic BP (mmHg)	109 ± 10	110 ± 11	110 ± 11	0.05
Diastolic BP (mmHg)	68 ± 9	69 ± 9	68 ± 10	0.25
BMI (kg/m ²)	23.6 ± 3.9	24.2 ± 4.4	24.9 ± 5.2	<0.01
Total Cholesterol (mg/dL)	177.7 ± 33.3	176.4 ± 32.0	178.4 ± 33.3	0.38
HDL Cholesterol (mg/dL)	54.4 ± 12.9	53.6 ± 13.1	52.3 ± 12.5	<0.01
Smoking (%)	16.4	23.7	38.0	<0.01
Anti-HTN Tx (%)	1.87	2.27	2.38	0.70
Diabetes (%)	0.56	0.39	0.99	0.21
CRP at Y7, ug/ml	2.07 ± 3.91	2.56 ± 4.07	3.36 ± 4.07	<0.01
CRP at Y15, ug/ml	2.65 ± 5.16	2.84 ± 3.81	3.90 ± 5.90	<0.01
CRP at Y20, ug/ml	2.31 ± 4.62	2.58 ± 4.07	3.31 ± 5.54	<0.01
Fibrinogen at Y5, mg/dl	251.6 ± 52.7	262.5 ± 55.5	271.6 ± 61.0	<0.01
Fibrinogen at Y20, mg/dl	393.4 ± 84.3	404.3 ± 90.7	415.4 ± 95.6	<0.01
IL-6 at Y20, pg/ml	2.48 ± 3.10	2.50 ± 2.90	2.77 ± 2.90	0.05

Baseline characteristics of participants across sex-adjusted leukocyte count quartiles (first to fourth: Q1 to Q4).

	<i>Q1</i>	<i>Q2</i>	<i>Q3</i>	<i>Q4</i>	<i>P-value</i> ^a
Participants, n	1085	1113	1069	1110	
Leukocyte counts, cells x 10 ⁹ /L - median [percentile 25 th –75 th]	4.74 [4.30–5.06]	5.84 [5.60–6.10]	6.90 [6.60–7.15]	8.50 [7.90–9.40]	NA
Age, years - mean ± SD	67.2 ± 6.1	67.1 ± 6.1	67.1 ± 6.2	66.9 ± 6.3	0.711
Sex,					
Women, % (n)	57.1 (619)	55.7 (620)	57.7 (617)	56.3 (625)	NA
Body Mass Index, kg/m ² - mean ± SD	29.5 ± 3.7	29.9 ± 3.5	30.1 ± 3.7	30.3 ± 3.9	<0.001
Waist circumference, cm - mean ± SD	98.6 (10.1)	99.5 (10.4)	100.4 (10.0)	101.1 (10.2)	<0.001
Current smoker, % (n)	7.8 (85)	11.1 (124)	15.2 (163)	24.1 (268)	<0.001
Former smoker, % (n)	24.1 (262)	27.9 (310)	25.4 (271)	23.2 (257)	0.064
Physical activity, METs - mean ± SD	263 ± 267	246 ± 242	247 ± 267	218 ± 225	< 0.001
Mediterranean diet score, 0 to 14 points - mean ± SD	8.8 ± 1.9	8.7 ± 2.0	8.7 ± 1.9	8.6 ± 1.9	0.052
Systolic blood pressure, mmHg - mean ± SD	147.6 ± 19.3	148.9 ± 20.2	150.0 ± 20.5	151.4 ± 20.9	<0.001
Diastolic blood pressure, mmHg - mean ± SD	82.4 ± 10.9	83.2 ± 10.5	83.1 ± 10.4	83.8 ± 11.1	0.026
Fasting plasma glucose, mg/dL - mean ± SD	114.0 ± 35.7	120.0 ± 40.7	124.0 ± 42.0	127.1 ± 45.8	<0.001
Triglycerides, mg/dL [percentile 25 th –75 th]	104.0 [77.0–142.0]	120.0 [88.0–163.0]	124.5 [94.0–168.0]	132.0 [100.0–178.7]	<0.001
HDL-cholesterol, mg/dL [percentile 25 th –75 th]	55.0 [47.0–67.0]	53.0 [45.0–62.9]	52.0 [44.0–62.0]	59.0 [43.0–59.0]	<0.001
Prevalence of type 2 diabetes mellitus, % (n)	40.1 (435)	44.0 (490)	51.2 (547)	52.0 (577)	<0.001
Metabolic syndrome and prevalence of its components					
Metabolic syndrome, % (n)	51.7 (561)	60.1 (669)	65.7 (702)	71.8 (808)	<0.001
Abdominal obesity, % (n)	68.6 (737)	71.7 (791)	74.9 (796)	74.9 (828)	0.002
Hypertriglyceridemia, % (n)	24.1 (261)	32.9 (365)	34.6 (369)	40.5 (448)	<0.001
Low HDL-cholesterol level, % (n)	21.2 (229)	25.8 (286)	28.6 (305)	32.9 (362)	<0.001
Hypertension, % (n)	93.5 (1015)304	94.0 (1045)	94.0 (1005)	96.3 (1067)	0.020
High fasting plasma glucose concentrations, % (n)	56.8 (616)	63.0 (701)	67.4 (720)	71.2 (790)	<0.001

White Blood Cell Counts as Risk Markers of Developing Metabolic Syndrome and Its Components in the Predimed Study

Nancy Babio^{1,2}, Núria Ibarrola-Jurado^{1,2}, Mònica Bulló^{1,2}, Miguel Ángel Martínez-González³, Julia Wärnberg⁴, Itziar Salaverria^{5,6}, Manuel Ortega-Calvo², Ramón Estruch^{2,7}, Lluís Serra-Majem⁸, Maria

Incidence of new cases of metabolic syndrome during the follow-up among subjects initially free of metabolic syndrome according to baseline sex-adjusted leukocyte counts.

	Q1n= 399	Q2n= 409	Q3n= 410	Q4n= 399	P-value
Total leukocytes					
Metabolic syndrome incidence, %	39.0	45.1	45.0	50.0	0.020 ^a
Unadjusted	1	1.29 (0.97–1.70)	1.28 (0.97–1.69)	1.56 (1.18–2.07)	0.003 ^b
Adjusted model 1 ^c	1	1.28 (0.97–1.69)	1.26 (0.95–1.67)	1.54 (1.15–2.05)	0.006 ^b
Adjusted model 2 ^d	1	1.22 (0.92–1.63)	1.22 (0.92–1.62)	1.53 (1.12–2.02)	0.009 ^b
Fully-adjusted model ^e	1	1.19 (0.88–1.59)	1.14 (0.85–1.53)	1.37 (1.01–1.86)	0.062 ^b
Neutrophils					
Metabolic syndrome incidence, %	41.4	40.5	44.4	57.7	0.005 ^a
Unadjusted	1	0.97 (0.73–1.28)	1.13 (0.86–1.50)	1.52 (1.15–2.01)	<0.001 ^b
Adjusted model1 ^c	1	0.95 (0.85–1.48)	1.12 (0.85–1.48)	1.49 (1.12–2.00)	0.001 ^b
Adjusted model 2 ^d	1	0.97 (0.73–1.29)	1.12 (0.84–1.49)	1.47 (1.09–1.96)	0.002 ^b
Fully-adjusted model ^e	1	0.89 (0.66–1.20)	1.04 (0.77–1.39)	1.32 (0.98–1.80)	0.004 ^b

Competing Interests: The authors have declared that no competing interests exist.

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† The PREDIMED Study Investigators are provided in the Acknowledgments.

Introduction

In recent years, the prevalence of metabolic syndrome (MetS) has increased dramatically in both developed and developing

countries. MetS is a constellation of cardiovascular risk factors (impaired glucose tolerance, hypertension, dyslipidemia and central obesity) strongly associated with type 2 diabetes, cardiovascular diseases, and other chronic conditions, all of which

White Blood Cell Counts as Risk Markers of Developing Metabolic Syndrome and Its Components in the Predimed Study

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Prevalence of metabolic syndrome for sex-adjusted quartiles of total white blood cell count and leukocyte subtypes.

	Q1n=1085	Q2n=1113	Q3n=1069	Q4n=1110	P for trend
Total leukocytes					
Cells x 10 ⁹ /L- median [percentile 25 th –75 th]	4.74 [4.30–5.06]	5.84 [5.60–6.10]	6.90 [6.60–7.15]	8.50 [7.90–9.40]	NA
Unadjusted	1	1.41 (1.19–1.67)	1.79 (1.50–2.13)	2.29 (1.91–2.73)	< 0.001
Adjusted ^a	1	1.35 (1.12–1.61)	1.70 (1.42–2.05)	2.47 (2.03–2.99)	< 0.001
Neutrophils					
Cells x 10 ⁹ /L- median [percentile 25 th –75 th]	2.53 [2.20–2.89]	3.22 [2.86–3.60]	3.90 [3.52–4.34]	5.12 [4.43–5.90]	NA
Unadjusted	1	1.23 (1.03–1.45)	1.65 (1.39–1.96)	2.21 (1.85–2.64)	< 0.001
Adjusted ^a	1	1.22 (1.02–1.47)	1.66 (1.38–2.00)	2.09 (1.73–2.54)	< 0.001

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Introduction

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countries. MetS is a constellation of cardiovascular risk factors (impaired glucose tolerance, hypertension, dyslipidemia and central obesity) strongly associated with type 2 diabetes, cardiovascular diseases, and other chronic conditions, all of which

Research Article

Associations between White Blood Cell Count and the Development of Incidental Nonalcoholic Fatty Liver Disease

Q1: ≤ 4300 , Q2: 4310–5040, Q3: 5050–6030, and Q4: ≥ 6040 cells/ μ L for women and
 Q1: ≤ 4710 , Q2: 4720–5500, Q3: 5510–6455, and Q4: ≥ 6460 cells/ μ L for men.

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Women (cells/ μ L)	Q1 (N = 313)	Q2 (N = 313)	Q3 (N = 313)	Q4 (N = 313)	P for trend
Age, years	47.0 $\pm$ 9.2	48.0 $\pm$ 9.2	46.8 $\pm$ 9.3	46.6 $\pm$ 9.4	0.243
Body mass index, kg/m ²	21.2 $\pm$ 2.2	21.8 $\pm$ 2.4	21.9 $\pm$ 2.4	22.4 $\pm$ 2.9	<0.001
Waist circumference, cm	79.1 $\pm$ 6.7	79.8 $\pm$ 6.7	79.8 $\pm$ 6.9	80.8 $\pm$ 7.5	0.017
ALT, IU/L	18.8 $\pm$ 14.3	16.7 $\pm$ 8.6	17.4 $\pm$ 9.4	17.4 $\pm$ 10.1	0.090
Fasting glucose, mg/dL	92.4 $\pm$ 10.5	93.9 $\pm$ 8.8	95.5 $\pm$ 14.0	94.3 $\pm$ 12.6	0.010
Triglycerides, mg/dL	70.5 $\pm$ 29.9	84.1 $\pm$ 42.4	85.9 $\pm$ 43.2	91.6 $\pm$ 50.9	<0.001
HDL cholesterol, mg/dL	62.4 $\pm$ 13.8	60.7 $\pm$ 13.1	59.5 $\pm$ 13.1	56.6 $\pm$ 12.6	<0.001
White blood cell counts, cells/ μ L	37.8 $\pm$ 4.3	46.6 $\pm$ 2.1	54.8 $\pm$ 2.9	70.6 $\pm$ 8.9	<0.001
Current smoking, %	6 (1.9)	11 (3.5)	2 (0.6)	11 (3.5)	0.072
Hypertension, %	32 (10.2)	28 (8.9)	24 (7.7)	43 (13.7)	0.071
5 yr_NAFLD, %	29 (9.3)	55 (17.6)	58 (17.9)	78 (24.9)	<0.001
Men (cells/ μ L)	Q1 (N = 238)	Q2 (N = 239)	Q3 (N = 239)	Q4 (N = 239)	P for trend
Age, years	48.8 $\pm$ 9.3	50.5 $\pm$ 10.0	50.1 $\pm$ 10.2	49.1 $\pm$ 9.4	0.171
Body mass index, kg/m ²	23.3 $\pm$ 2.4	23.8 $\pm$ 2.3	24.3 $\pm$ 2.3	24.2 $\pm$ 2.3	<0.001
Waist circumference, cm	83.6 $\pm$ 6.5	85.4 $\pm$ 6.3	86.7 $\pm$ 6.3	86.5 $\pm$ 6.3	<0.001
ALT, IU/L	22.1 $\pm$ 13.1	22.3 $\pm$ 10.8	24.2 $\pm$ 10.0	25.2 $\pm$ 13.9	0.012
Fasting glucose, mg/dL	100.1 $\pm$ 13.8	102.2 $\pm$ 16.3	104.3 $\pm$ 17.8	104.7 $\pm$ 21.5	0.020
Triglycerides, mg/dL	95.5 $\pm$ 47.0	114.8 $\pm$ 59.4	125.3 $\pm$ 84.1	142.6 $\pm$ 74.3	<0.001
HDL cholesterol, mg/dL	53.6 $\pm$ 11.8	49.9 $\pm$ 11.6	48.9 $\pm$ 10.3	46.9 $\pm$ 10.2	<0.001
White blood cell counts, cells/ μ L	41.8 $\pm$ 3.9	51.0 $\pm$ 2.1	59.4 $\pm$ 2.7	75.5 $\pm$ 8.5	<0.001
Current smoking, %	34 (14.7)	46 (19.7)	68 (28.8)	103 (44.0)	<0.001
Hypertension, %	32 (13.9)	47 (20.1)	46 (19.5)	51 (21.8)	0.145
5 yr_NAFLD, %	58 (25.1)	77 (32.9)	91 (38.6)	113 (48.3)	<0.001

White Blood Cell Count

Traditional Reference Range:

* $3.4 - 10.8 \times 10^3/\mu\text{L}$

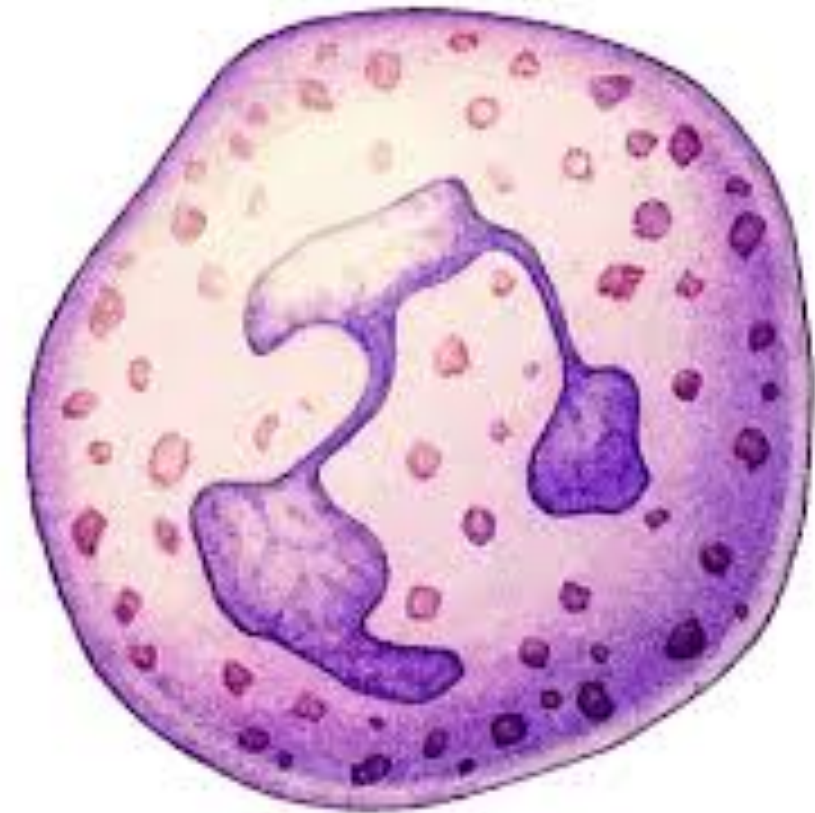
Optimal Reference Range

* $4.5 - 6.5 \times 10^3/\mu\text{L}$

Neutrophils

- * The most abundant and most motile of all white blood cells
- * Neutralizes an invader by phagocytosis
 - * Contains a number of enzymes and pyrogens that can also cause damage to host cells

Optimal range: 50-60%



Neutrophils

Elevated

- * Generally due to bacterial infection and/or inflammation

Decreased

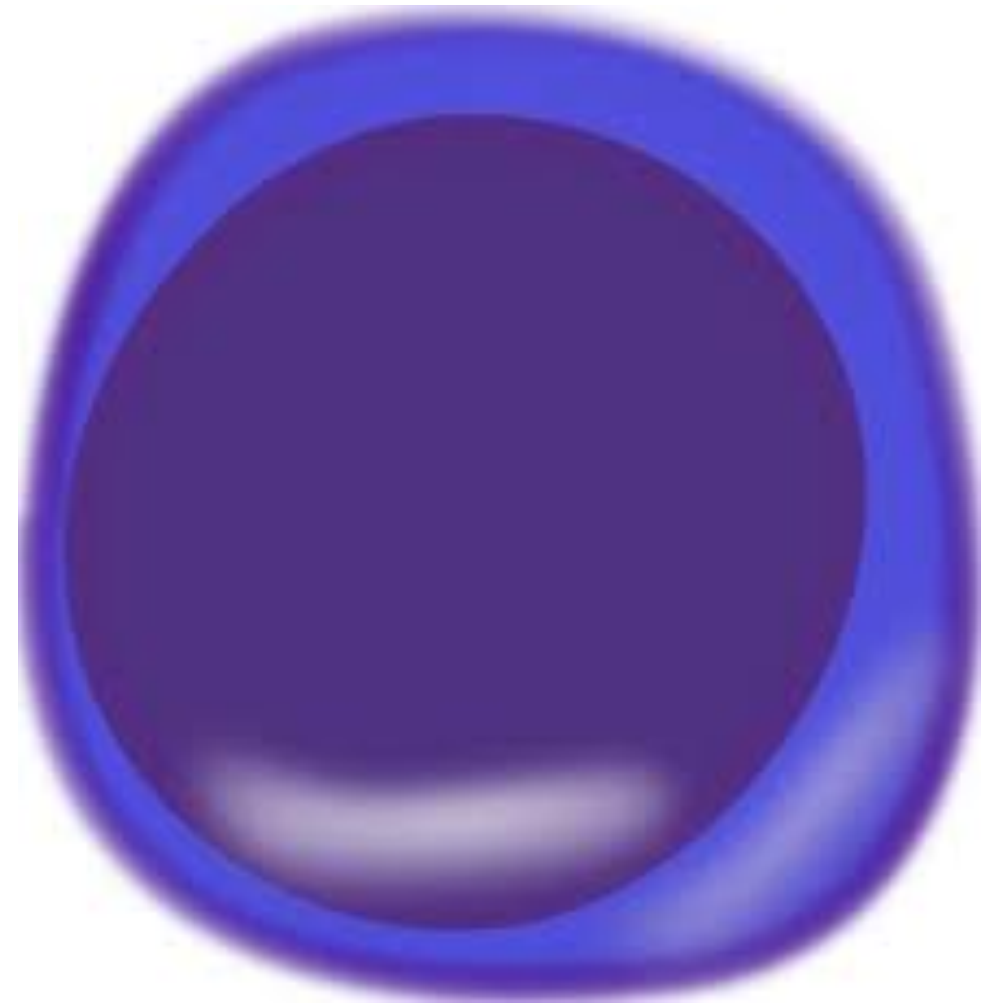
- * Copper deficiency
 - * Low hemoglobin/RBC, ferritin may be elevated, possible ataxia; high zinc intake (>50mg/day)
- * B12 or folate deficiency
- * Elevations in other types of white blood cells

Interfering factors: stress, excitement, fear, anger, joy and exercise temporarily cause increase neutrophils.

Lymphocytes

- * Lymphocytes contain a wide variety of white blood cells including B-cells, T-cells and Natural Killer Cells
- * The lymphocyte count does not differentiate between these – more specific testing is required to identify the lymphocyte subsets

Optimal range: 30-35%



Lymphocytes

Elevated

- * Viral infections
- * Crohn's disease
- * Other autoimmune diseases
- * Hypoadrenalism (cortisol suppresses lymphocytes, so low cortisol may cause elevation – check sodium and potassium)

Decreased

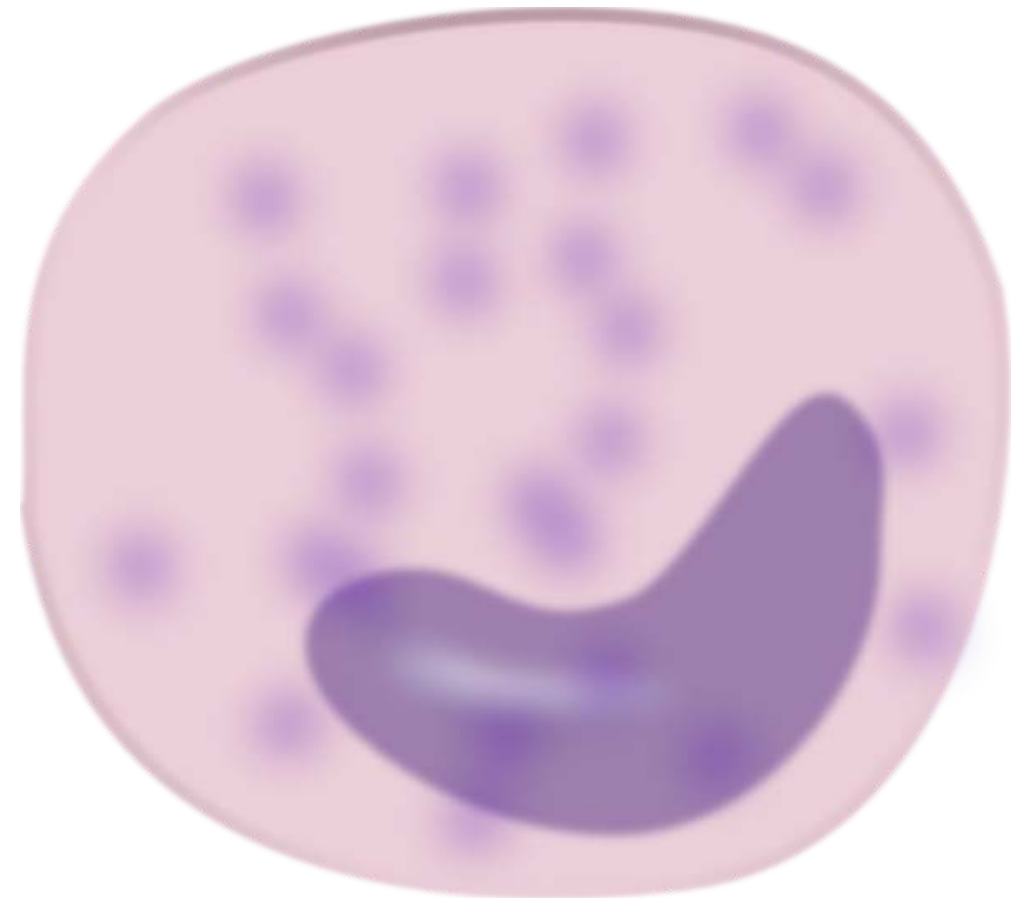
- * Zinc deficiency
- * Elevations of other white blood cells

Interfering factors: Exercise, emotional stress and menstruation can cause increase in lymphocytes.

Monocytes

- * Monocytes leave circulation within 16-36 hours and become macrophages
- * Macrophages remove foreign substances via phagocytosis
 - * They also participate in the destruction of old red blood cells, denatured proteins, microorganisms, and dead cells
- * Macrophages can become Antigen Presenting Cells (APCs) and deliver an antigen to lymphocytes

Optimal range: <6%



Monocytes

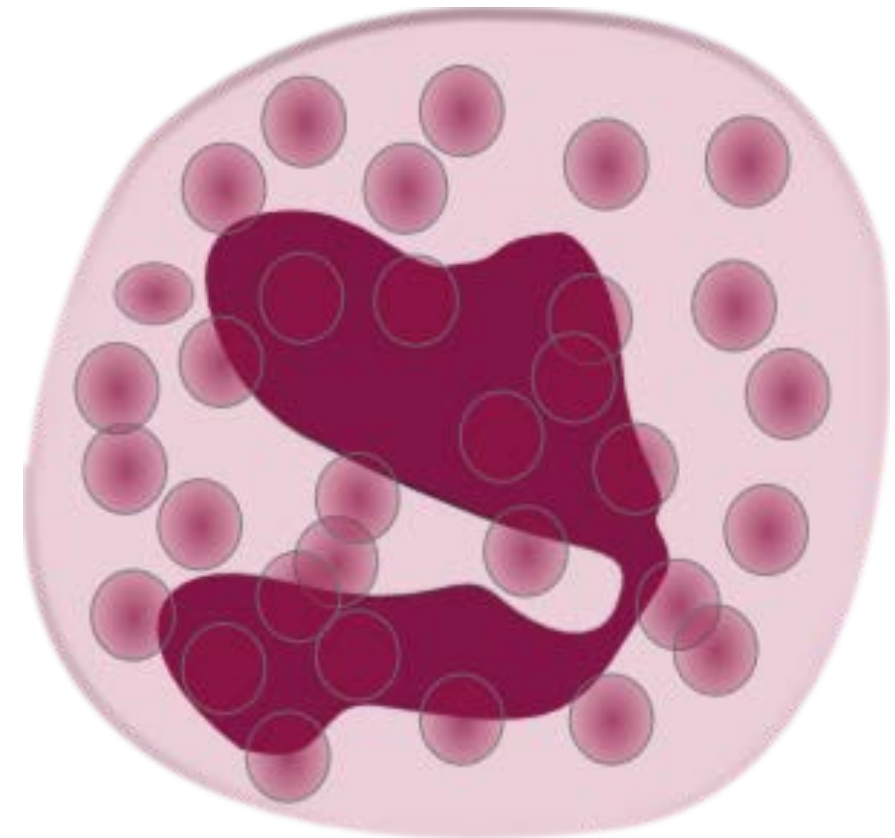
Elevated

- * Generalized, systemic inflammation
- * Collagen disease, such as Rheumatoid Arthritis
- * Ulcerative colitis
- * Recovery state after an infection or trauma
- * One study showed that a white blood cell count of 5.5 with high-normal monocytes (6.1%) was associated with non-alcoholic fatty liver disease
 - * Kim, Hack-Lyoung, Goh Eun Chung, In Young Park, Jin Man Choi, Se-Min Hwang, Jeong-Hoon Lee, and Donghee Kim. 2011. "Elevated Peripheral Blood Monocyte Fraction in Nonalcoholic Fatty Liver Disease." *The Tohoku Journal of Experimental Medicine* 223 (3): 227–33.

Eosinophils

- * Eosinophils are also phagocytic immune cells and become active in the later stages of inflammation.
- * They are found in large numbers in the intestines and lungs.

Optimal range: <3%



Eosinophils

- * One study suggested eosinophils may be elevated in primary biliary cirrhosis, which can also raise HDL levels.
 - * Thus if they are both elevated, consider primary biliary cirrhosis.
- * Eosinophil levels also tend to correlate with platelet counts and fibrinogen.

Eosinophils

Elevated

- * Allergies, asthma
- * Parasitic infection, tapeworms
- * Hypoadrenalism
- * Chronic skin disease, such as eczema
- * Ulcerative colitis, Crohn's
- * Aspirin sensitivity

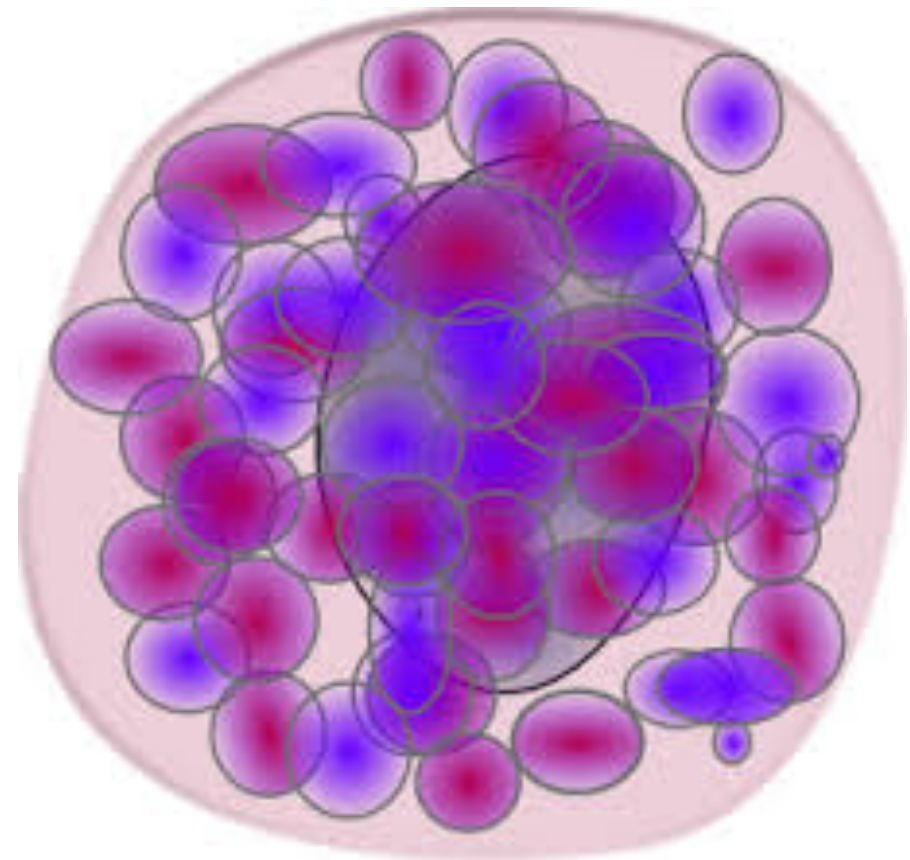
Decreased

- * Significantly elevated cortisol

Interfering factors: Lowest in the morning. Stress and high cortisol can lower numbers.

Basophils

- * Basophils contain a large number of granules containing heparin, histamine, leukotriene, serotonin, etc.
- * They are associated with inflammation and hypersensitivities
- * Tissue basophils are called mast cells.



Basophils

Elevated

- * Inflammation, allergy
- * Chronic hemolytic anemia
- * Hypothyroidism

Platelet Count

- * Platelets, also known as thrombocytes, are small, anucleated, cell fragments found in the blood.
- * Their lifespan is about 7-10 days.
- * Platelets are involved in the clotting cascade and contain a number of chemicals that they release, when activated, that promotes clotting.
- * They are also necessary for vascular integrity and vasoconstriction
- * Low levels of platelets can cause increased bleeding; elevated levels can increase clotting

Blood platelet count and function are related to total and cardiovascular death in apparently healthy men.

E Thaulow, J Erikssen, L Sandvik, H Stormorken and P F Cohn

TABLE 1. Relation Between Total Platelet Counts and Annual Coronary Heart Disease and Total Mortality in 487 Apparently Healthy Middle-Aged Men Followed Up for 13.5 Years

	Quartile			
	1	2	3	4
CHD deaths (%)	0.36	0.41	0.44	1.04
Total deaths (%)	0.73	0.94	0.89	1.53
Median platelet				
Count ($10^9/l$)	176	211	239	298
<i>n</i>	123	126	117	121

10% of the 487 men with the highest platelet counts (mean platelet count 305) had an annual CHD mortality of 1.27%.

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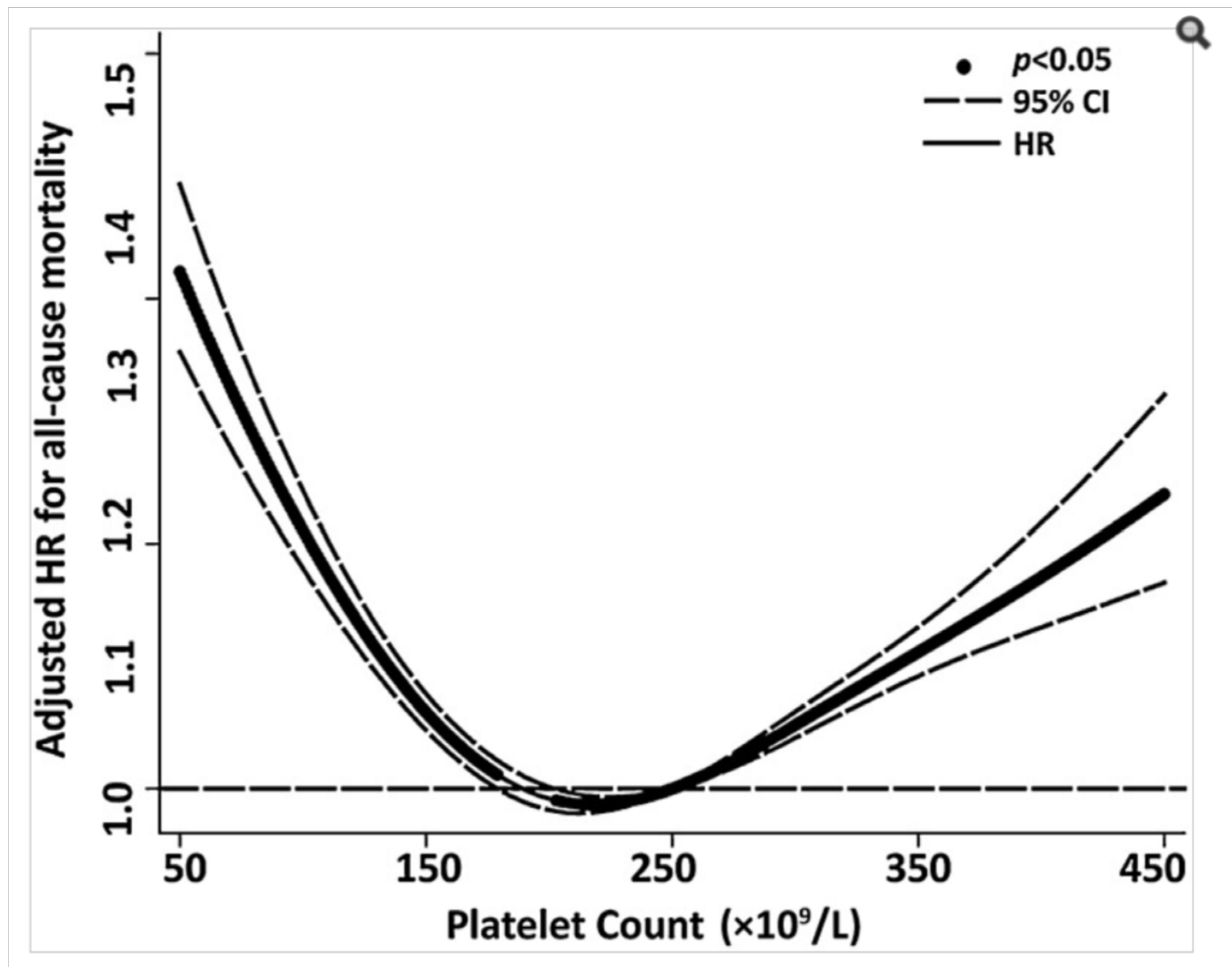
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Incidence and risks of all-cause and cause-specific mortality in older people

Platelet count, ×10 ⁹ /L	No. of events	No. of person-years	Incidence rate [*]	Cox proportional hazard models			
				Crude HR (95% CI)	<i>P</i>	Adjusted HR [‡] (95% CI)	<i>P</i>
All-cause mortality							
<100	602	10 730	56.10	2.80 (2.58-3.04)	<.001	1.93 (1.77-2.11)	<.001
100 to <200	7598	304 486	24.95	1.25 (1.21-1.29)	<.001	1.08 (1.05-1.12)	<.001
200 to <300	7827	393 530	19.89	1		1	
300 to <400	1384	52 724	26.25	1.33 (1.26-1.41)	<.001	1.18 (1.11-1.25)	<.001
≥400	348	5 987	58.13	3.00 (2.69-3.33)	<.001	1.67 (1.49-1.87)	<.001
Cardiovascular mortality [‡]							
<100	72	9 256	7.78	1.78 (1.41-2.26)	<.001	1.24 (0.97-1.59)	.086
100 to <200	1405	267 048	5.26	1.20 (1.12-1.29)	<.001	1.06 (0.98-1.14)	.151
200 to <300	1529	350 235	4.37	1		1	
300 to <400	215	47 125	4.56	1.05 (0.91-1.21)	.511	0.96 (0.82-1.11)	.568
≥400	50	5 233	9.55	2.17 (1.63-2.89)	<.001	1.38 (1.02-1.86)	.037

* Tsai, Ming-Tsun, Yung-Tai Chen, Chi-Hung Lin, Tung-Po Huang, Der-Cherng Tarng, and Taiwan Geriatric Kidney Disease Research Group. 2015. "U-Shaped Mortality Curve Associated with Platelet Count among Older People: A Community-Based Cohort Study." *Blood* 126 (13): 1633–35. doi:10.1182/blood-2015-06-654764.



- * Tsai, Ming-Tsun, Yung-Tai Chen, Chi-Hung Lin, Tung-Po Huang, Der-Cherng Tarng, and Taiwan Geriatric Kidney Disease Research Group. 2015. "U-Shaped Mortality Curve Associated with Platelet Count among Older People: A Community-Based Cohort Study." *Blood* 126 (13): 1633–35. doi:10.1182/blood-2015-06-654764.

Platelet Count

Traditional Reference Range

* $140-400 \times 10^3/\text{mm}^3$

Optimal Reference Range

* $225-275 \times 10^3/\text{mm}^3$

Platelet Count - Increased

Cause	Reason	Additional Inquiry
Iron deficiency anemia	Mechanism unknown. Possibly due to increased erythropoietin levels.	*Should be noted that some references show low platelets in severe iron deficiency anemia.
Collagen diseases (rheumatoid arthritis, lupus)		
Hemolytic anemia and/or blood loss	Increased production of all blood cells	Evaluate CBC.
Stress, infection, inflammation	Increased thrombosis during inflammation.	

- * In 50% of patients who exhibit unexpected platelet increases, a malignancy is found. But this must be outside of the laboratory reference range.

Platelet count - Decreased

Cause	Reason	Additional Inquiry
Alcoholism	Poor liver function, leading to decreased thrombopoetin.	Evaluate liver markers.
Liver dysfunction	The liver produces thrombopoetin, which stimulates platelet production. Therefore poor liver function can lead to low thrombopoetin levels.	Evaluate liver markers.
Certain viral and bacterial infections	Observation.	Evaluate WBC, lymphocytes.
Pernicious anemia	Poor nuclear function, including megakariocytes.	
Bleeding	Blood loss can lead to decrease of all blood cells, including platelets.	Client history. CBC.

Ratios

- * Neutrophil/Lymphocyte Ratio
 - * < 2.4
- * Platelet/Lymphocyte Ratio
 - * < 140
 - * Platelets 238/lymphocytes 1.7 = 140
- * Lymphocyte/Monocyte Ratio
 - * > 4
 - * Lymphocyte 2.0/monocytes .5 = 4



Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio are Predictors of Heart Failure

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Abstract

Background: Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are inflammatory markers used as prognostic factors in various diseases.

Objectives: To compare the PLR and the NLR of heart failure (HF) patients with those of age-sex matched controls, to evaluate the predictive value of those markers in detecting HF, and to demonstrate the effect of NLR and PLR on mortality in HF patients during follow-up.

Methods: This study included 56 HF patients and 40 controls without HF. All subjects underwent transthoracic

NLR and PLR were higher in HF patients than in age-sex matched controls. However, NLR and PLR were not sufficient to establish a diagnosis of HF.

The best cut-off value of NLR to predict HF was 3.0, with 86.3% sensitivity and 77.5% specificity, and the best cut-off value of PLR to predict HF was 137.3, with 70% sensitivity and 60% specificity.

aging of the population and the decreased cardiac mortality and morbidity related to modern treatment approaches worldwide. The prevalence of HF is approximately 10% in patients aged over 70 years¹. Despite advanced treatment approaches, mortality rates are still high. Moreover, the need for long-term care of HF patients constitutes a difficult situation for caregivers and relatives of patients.

Previous studies have shown that inflammation plays an important role in the initiation and progression of cardiovascular diseases (CVD)²⁻⁴. Chronic inflammation is also more common in HF patients⁴. White blood cells (WBCs) and their subtypes

ratio (PLR) are novel inflammatory biomarkers used as prognostic factors in various diseases^{5,10,11}. Previous studies have demonstrated that a higher NLR is associated with a higher mortality rate in those with coronary artery disease (CAD)^{5,6,10}. A study carried out by Uthamalingam et al⁶ showed that a higher NLR is associated with higher mortality in HF patients. However, to the best of our knowledge, there is no data about the significance of the PLR in HF patients in the literature. Comparison of the PLR of HF patients with those of age-sex matched healthy controls has not been studied previously. Therefore, the first aim of this study was to compare the PLR and the NLR of HF patients with those of age-sex matched controls. The second aim was to evaluate the predictive value of NLR and PLR in detecting HF. The third aim was to demonstrate the effect of the PLR and the NLR on mortality in HF patients during follow-up.

Methods

Study population

Between June 2012 and October 2013, 71 patients diagnosed with decompensated HF were evaluated for study

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DOI: 10.5935/abc.20150126

The platelet-to-lymphocyte ratio as a predictor of all-cause mortality in patients with coronary artery disease undergoing elective percutaneous coronary intervention and stent implantation



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Mortality was highest in patients with PLR within the 3rd tertile as compared to the 2nd and the 1st tertile.

Group I included patients with PLR < 87 ($n = 986$); group II included patients with PLR value of ≥ 87 and < 121 ($n = 986$); and group III included patients with PLR ≥ 121 ($n = 987$).

Conclusion: PLR has potential predictive value in patients with SCAD, which has not been reported previously, but statistical significance disappears after adjusting for estimated glomerular filtration rate (eGFR) and hemoglobin levels as a potential confounding variable.

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Keywords: Platelet-to-lymphocyte ratio, Percutaneous coronary intervention, Mortality

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Prognostic role of lymphocyte to monocyte ratio for patients with cancer: evidence from a systematic review and meta-analysis

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*These authors contributed equally to this work.

Low lymphocyte to monocyte ratio (LMR) has been reported to be a poor prognostic indicator in several malignancies.

Fifty-six studies comprising 20,248 patients were included in the analysis. Overall, decreased LMR was significantly associated with shorter [onset of symptoms] in non-hematological malignancy and hematological malignancy.

By these results, we conclude that a decreased LMR implied poor prognosis in patients with cancer and could serve as a readily available and inexpensive biomarker for clinical decision.

Inflammatory responses play crucial roles at different stages of cancer development and progression and may be linked with systemic inflammation [1–3]. There is increasing evidence that systemic inflammatory response is a key determinant of outcome in patients with cancer, which is reflected by many biochemical or hematological parameters, such as increased C-reactive protein (CRP) levels, hypoalbuminemia or elevated white cell, neutrophil and platelet counts [4]. Several of these parameters have been converted to ratios or prognostic scores such as the neutrophil to lymphocyte ratio (NLR)

of CRP and albumin) [6].

Recently, a decreased ratio of peripheral lymphocyte to monocyte ratio (LMR) has been identified as a poor prognostic indicator in various cancers [7–12], which might be a readily available and inexpensive objective prognostic index that could be used to precisely guide clinical decisions. The LMR might be a good reflection of cancer, lymphopenia that is a surrogate marker of weak immune response and an elevated monocyte count, standing for a microenvironment surrogate marker of high tumor burden. However, the consistency and magnitude of the prognostic impact of LMR remain unclear. Therefore, we performed a

Ratios

- * Neutrophil/Lymphocyte Ratio
 - * < 2.4
- * Platelet/Lymphocyte Ratio
 - * < 140
 - * Platelets 238/lymphocytes 1.7 = 140
- * Lymphocyte/Monocyte Ratio
 - * > 4

Usefulness of a Complete Blood Count-Derived Risk Score to Predict Incident Mortality in Patients With Suspected Cardiovascular Disease

Jeffrey L. Anderson, MD*, Brianna S. Ronnow, MS, Benjamin D. Horne, PhD, MPH, John F. Carlquist, PhD, Heidi T. May, MSPH, Tami L. Bair, BS, Kurt R. Jensen, MS, and Joseph B. Muhlestein, MD, for the Intermountain Heart Collaborative (IHC) Study Group

The complete blood cell (CBC) count is an inexpensive, frequently obtained blood test whose information content is potentially underused. We examined the predictive ability of the CBC count for incident death in 29,526 consecutive consenting patients who underwent coronary angiography. Subjects were randomly assigned to training (60%) and test (40%) groups and were followed for an average of 4.9 years. Computed and integer risk score models for all-cause death were developed for 30 days and 1, 5, and 10 years using multivariable logistic regressions applied to CBC metrics, age, and gender. The study cohort was an average age of 61 years, 62% were men, and had a 3.3% annual risk of mortality. An integer (scalar) risk score (range 0 to 18) successfully separated patient cohorts into subgroups at markedly different mortality risks (<1% to >14% at 30 days).

Table 1
Quartiles of complete blood count components

Component	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Hematocrit (%)	≤38.8	38.9–42.2	42.3–45.3	≥45.4
WBC count ($\times 10^3/\mu\text{l}$)	≤6.0	6.1–7.4	7.5–9.4	≥9.5
Platelet count ($\times 10^3/\mu\text{L}$)	≤182	183–218	219–263	≥264
Mean corpuscular volume (fl)	≤86.7	86.8–89.8	89.9–92.7	≥92.8
Mean corpuscular hemoglobin concentration (g/dl)	≤33.6	33.7–34.1	34.2–34.5	≥34.6
Red cell distribution width (%)	≤12.6	12.7–13.2	13.3–14.0	≥14.1

counts are well recognized as risk factors for hemorrhage (with very low counts) or thrombosis (with very high counts), and specific platelet indexes have also been linked to cardiovascular risk.¹⁰ Although each CBC count parameter individually might provide modest predictive ability, a total CBC score, derived by combining elements from all 3 CBC count compartments (hemogram, leukocytes, and platelets), could have substantial advantages, but it has not

all-cause mortality in a moderate- to high-risk cardiovascular population at 30 days (primary time point), 1 year (secondary time point), and 5 and 10 years (exploratory time points). A second aim was to compare the predictive ability of the total CBC risk models[†] with simple models based on age, gender, and other standard risk factors and the single hemographic components, hematocrit, and WBC count.

Study population: baseline studies and follow-up: Consenting patients who underwent coronary angiography and entered into the catheterization laboratory registry of the Intermountain Heart Collaborative Study between 1993 and 2004 were studied if they had a baseline CBC measure-

Cardiovascular Department, LDS Hospital, and University of Utah, Salt Lake City, Utah. Manuscript received June 5, 2006; revised manuscript received and accepted August 1, 2006.

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E-mail address: jeffrey.anderson@intermountainmail.org (J.L. Anderson).

[†] A computerized CBC Risk Model program (© 2005 LDS Hospital, Salt Lake City, Utah) is available to all academic investigators by contacting Dr Anderson. Industry personnel may contact Heidi May at 801-408-2873 for licensing information.

Usefulness of a Complete Blood Count-Derived Risk Score to Predict Incident Mortality in Patients With Suspected Cardiovascular Disease

Jeffrey L. Anderson, MD*, Brianna S. Ronnow, MS, Benjamin D. Home, PhD, MPH

Table 3

Thirty-day complete blood cell count model and risk scores for all-cause mortality

Component (test vs referent Q)	Beta Coefficient ± SE	Hazard Ratio (95% CI)
Hematocrit		
Q1 vs Q4	0.699 ± 0.17‡	2.0 (1.5–2.8)
Q2 vs Q4	−0.159 ± 0.19	0.9 (0.6–1.3)
Q3 vs Q4	−0.097 ± 0.19	0.9 (0.6–1.3)
WBC count		
Q2 vs Q1	0.223 ± 0.23	1.3 (0.8–1.9)
Q3 vs Q1	0.600 ± 0.21†	1.8 (1.2–2.8)
Q4 vs Q1	1.653 ± 0.19‡	5.2 (3.6–7.6)
Platelet count		
Q1 vs Q4	0.373 ± 0.16*	1.5 (1.1–2.0)
Q2 vs Q4	−0.058 ± 0.17	0.9 (0.7–1.3)
Q3 vs Q4	−0.329 ± 0.18	0.7 (0.5–1.0)
Mean corpuscular volume		
Q2 vs Q1	0.242 ± 0.19	1.3 (0.9–1.8)
Q3 vs Q1	0.372 ± 0.18*	1.5 (1.0–2.1)
Q4 vs Q1	0.636 ± 0.17‡	1.9 (1.4–2.6)
Mean corpuscular hemoglobin concentration		
Q1 vs Q4	0.516 ± 0.19†	1.7 (1.1–2.5)
Q1 vs Q4	0.376 ± 0.19	1.5 (1.0–2.1)
Q1 vs Q4	0.097 ± 0.21	1.1 (0.7–1.7)
Red cell distribution width		
Q2 vs Q1	0.215 ± 0.20	1.2 (0.8–1.8)
Q2 vs Q1	0.222 ± 0.19	1.3 (0.9–1.8)
Q2 vs Q1	0.563 ± 0.19†	1.8 (1.2–2.5)
Male gender	0.074 ± 0.13	1.1 (0.8–1.4)
Age (yrs)		
≤29 vs 40–49	0.142 ± 0.64	1.2 (0.3–4.0)
30–39 vs 40–49	0.169 ± 0.48	1.2 (0.5–3.1)
50–59 vs 40–49	0.158 ± 0.30	1.2 (0.7–2.1)
70–79 vs 40–49	1.073 ± 0.27‡	2.9 (1.7–4.9)
≥80 vs 40–49	1.381 ± 0.28‡	4.0 (2.3–6.9)

* p < 0.05; † p < 0.01; ‡ p < 0.001.

CI = confidence interval; Q = quartile.

Table 4

One-year complete blood cell count Model and risk scores for all-cause mortality

Component (test vs referent Q)	Beta Coefficient ± SE	Hazard Ratio (95% CI)
Hematocrit		
Q1 vs Q4	0.889 ± 0.12‡	2.4 (1.9–3.1)
Q2 vs Q4	0.217 ± 0.13	1.2 (1.0–1.6)
Q3 vs Q4	0.016 ± 0.14	1.0 (0.8–1.3)
WBC count		
Q2 vs Q1	0.296 ± 0.13*	1.3 (1.0–1.7)
Q2 vs Q1	0.470 ± 0.13‡	1.6 (1.2–2.1)
Q2 vs Q1	1.161 ± 0.12‡	3.2 (2.5–4.0)
Platelet count		
Q1 vs Q4	0.478 ± 0.11‡	1.6 (1.3–2.0)
Q2 vs Q4	0.135 ± 0.12	1.1 (0.9–1.4)
Q3 vs Q4	−0.081 ± 0.12	0.9 (0.7–1.2)
Mean corpuscular volume		
Q2 vs Q1	0.017 ± 0.12	1.0 (0.8–1.3)
Q3 vs Q1	0.123 ± 0.12	1.1 (0.9–1.4)
Q4 vs Q1	0.301 ± 0.11†	1.4 (1.1–1.7)
Mean corpuscular hemoglobin concentration		
Q1 vs Q4	0.571 ± 0.13‡	1.8 (1.4–2.3)
Q1 vs Q4	0.175 ± 0.14	1.2 (0.9–1.6)
Q1 vs Q4	0.040 ± 0.14	1.0 (0.8–1.4)
Red cell distribution width		
Q2 vs Q1	0.310 ± 0.15*	1.4 (1.0–1.8)
Q2 vs Q1	0.356 ± 0.14*	1.4 (1.1–1.9)
Q2 vs Q1	1.098 ± 0.13‡	3.0 (2.3–3.9)
Male gender	0.069 ± 0.09	1.1 (0.9–1.3)
Age (yrs)		
≤29 vs 40–49	0.223 ± 0.43	1.2 (0.5–2.9)
30–39 vs 40–49	0.309 ± 0.33	1.4 (0.7–2.6)
50–59 vs 40–49	0.247 ± 0.21	1.3 (0.8–1.9)
60–69 vs 40–49	0.725 ± 0.20‡	2.1 (1.4–3.0)
70–79 vs 40–49	1.161 ± 0.19‡	3.2 (2.2–4.6)
≥80 vs 40–49	1.534 ± 0.20‡	4.6 (3.1–6.9)

* p < 0.05; † p < 0.01; ‡ p < 0.001.

Abbreviations as in Table 3.

Usefulness of a Complete Blood Count-Derived Risk Score to Predict Incident Mortality in Patients With Suspected Cardiovascular Disease

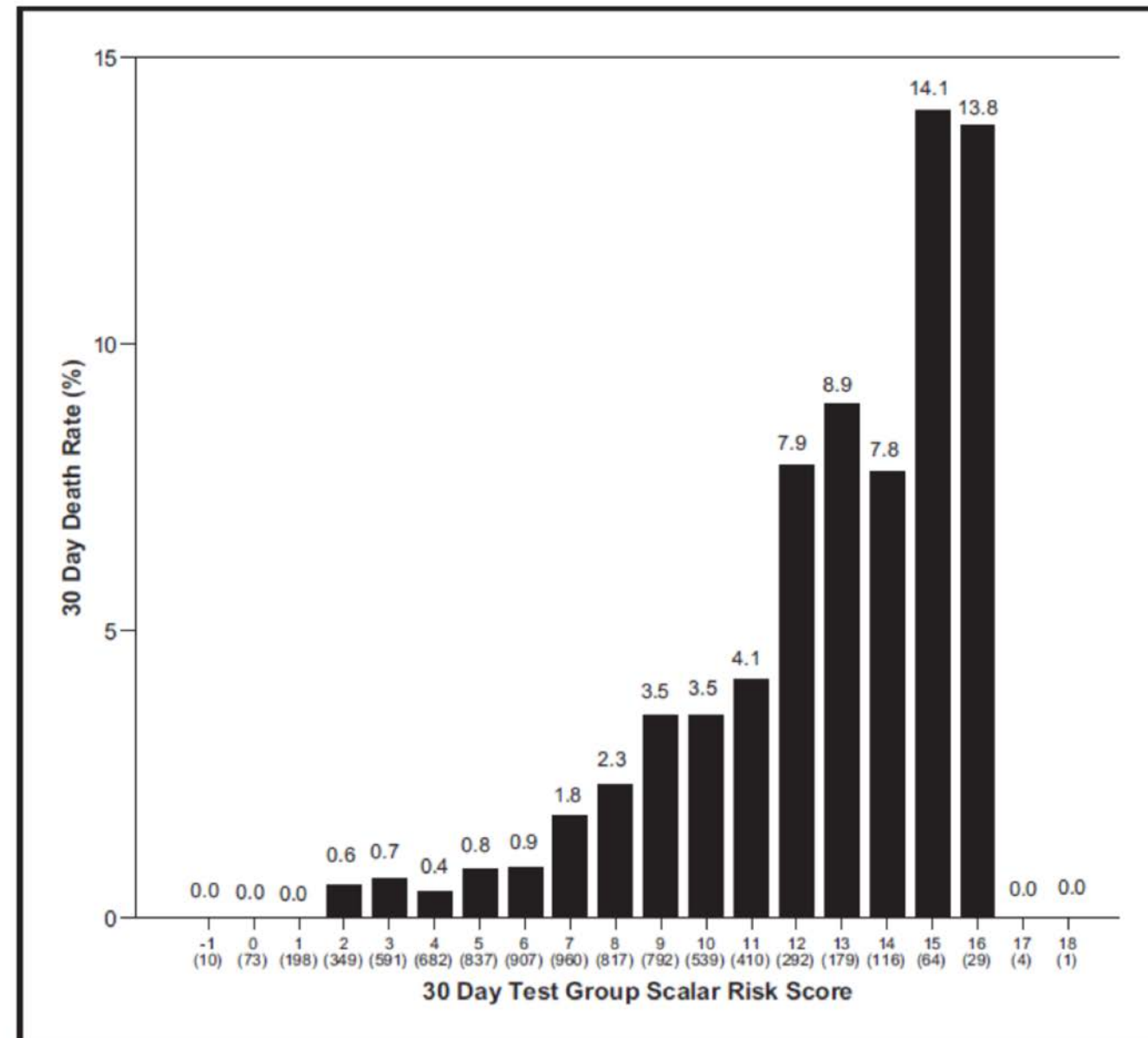
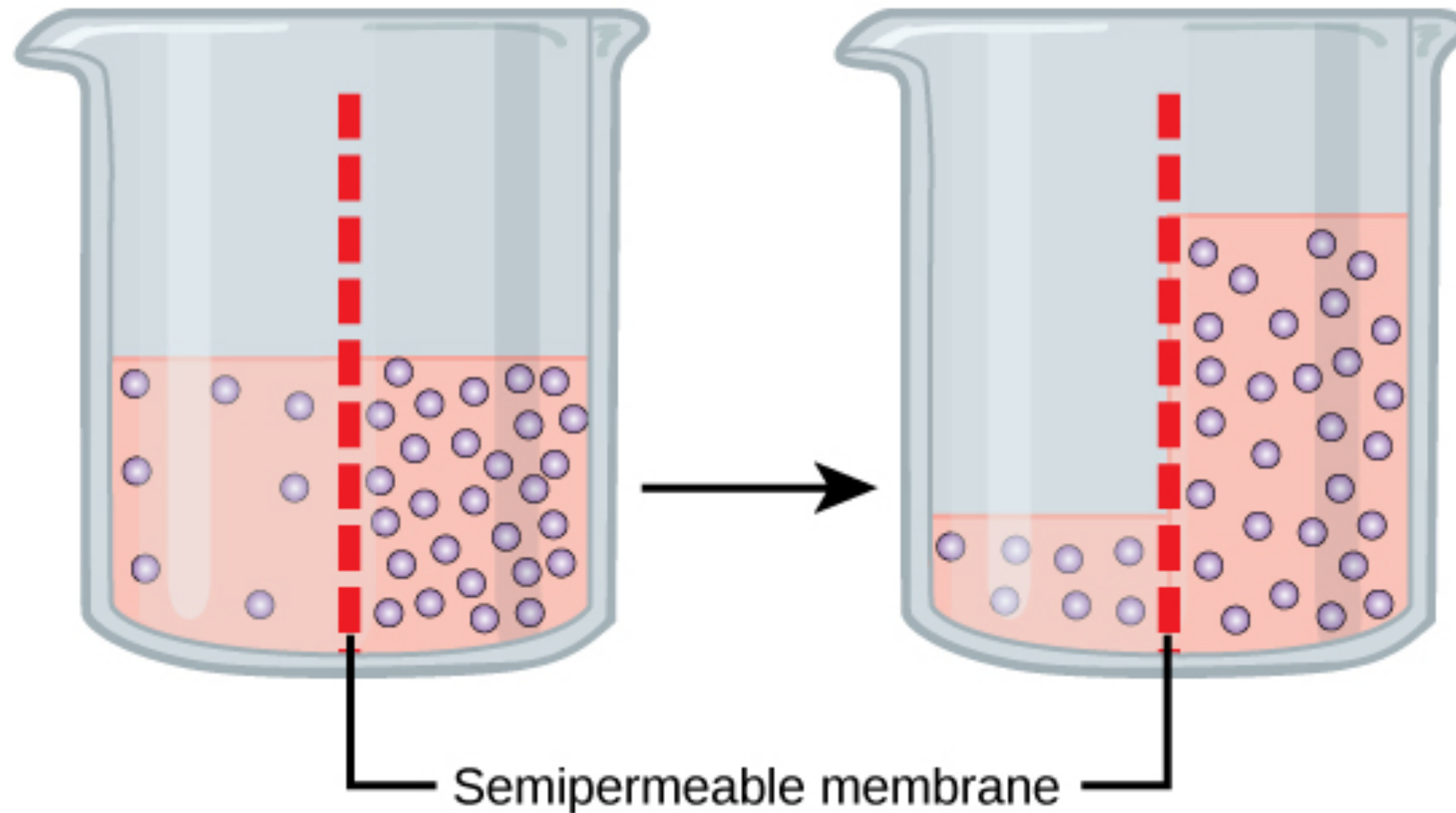


Figure 2. Thirty-day mortality by scalar risk score incorporating the 6 CBC count metrics plus age and gender for the test group. The number of patients within each risk score is presented in parentheses below each risk score. The percentage of death within each risk score is presented at the top of each bar. Cells with <20 patients were excluded.

Section Break Number 4

Electrolytes

Quick Word on Osmolarity



Electrolytes

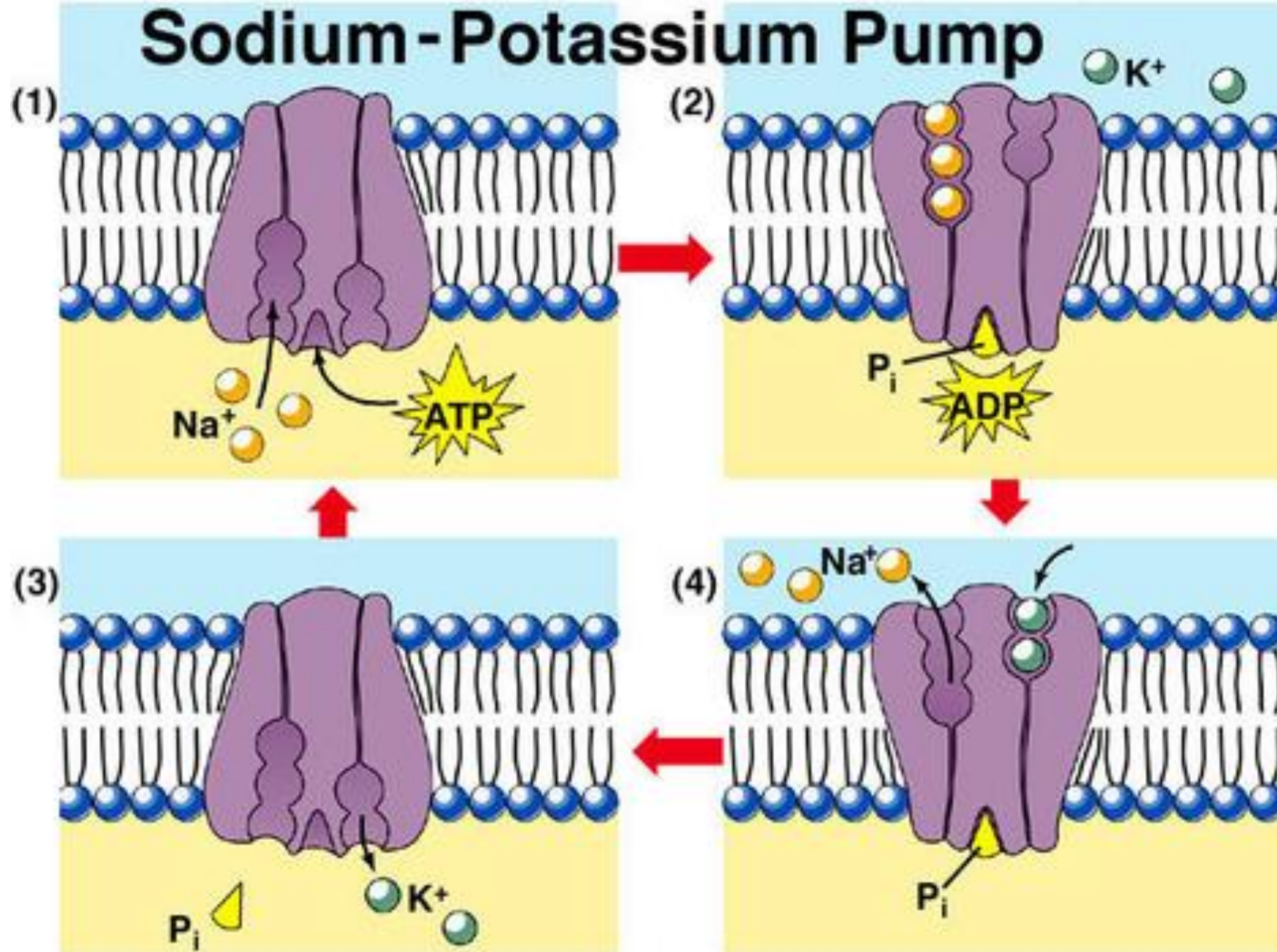
- * Sodium
- * Potassium
- * Magnesium
- * Chloride
- * CO₂ (bicarbonate)
- * Calcium
- * Phosphorus

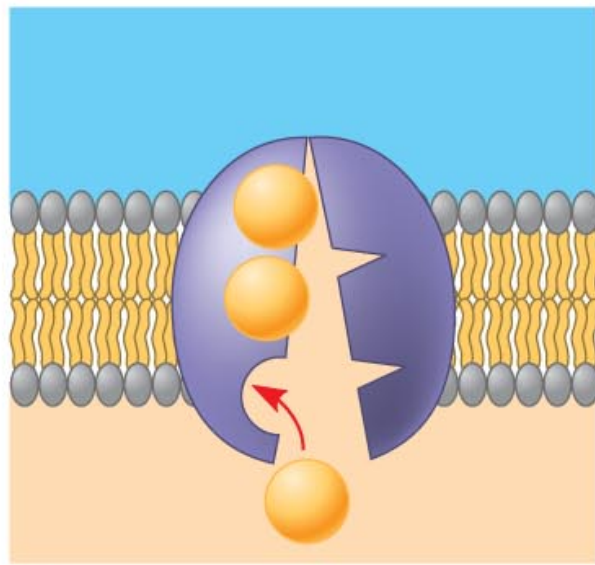
Sodium

- * Sodium is the most abundant extracellular cation (positively charged ion) in the body.
- * Though there is more calcium and phosphorus, it could be strongly argued that sodium is the most important cation due to its influences on fluid balance in the body.
- * Sodium has a critical role in overall electrolyte and water balance, including plasma volume, interstitial water volume and cellular hydration.
- * Sodium and electrolyte balance is necessary for proper muscular contraction, including the heart, absorption of nutrients in the GI tract, nerve impulse and action potentials, pH balance, and more.

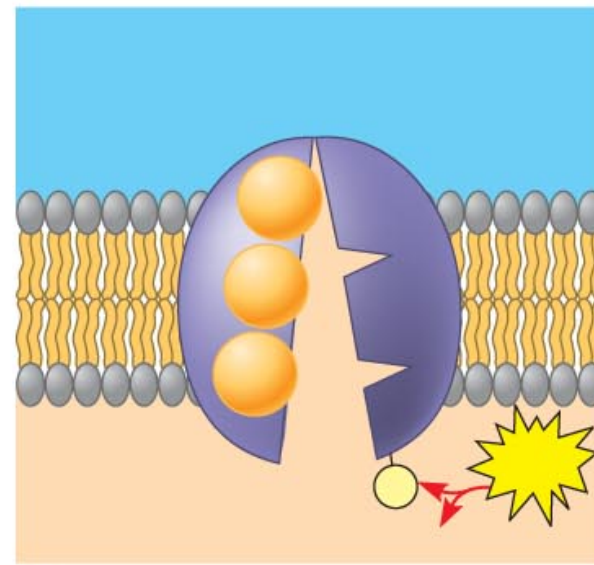
Sodium - Functions

- * Sodium helps achieve these via the NaKATPase pump or transporter (aka the Sodium-Potassium ATPase, or the Sodium-Potassium Pump).
- * It is worth noting that the NaKATPase pump requires ATP (eg energy) and as such is a substantial portion of the body's total metabolic activity and contributor to thermogenesis.
- * Fluid balance (osmotic gradient)
- * Nerve transmission (action potentials)
- * Muscle contraction (depolarization)

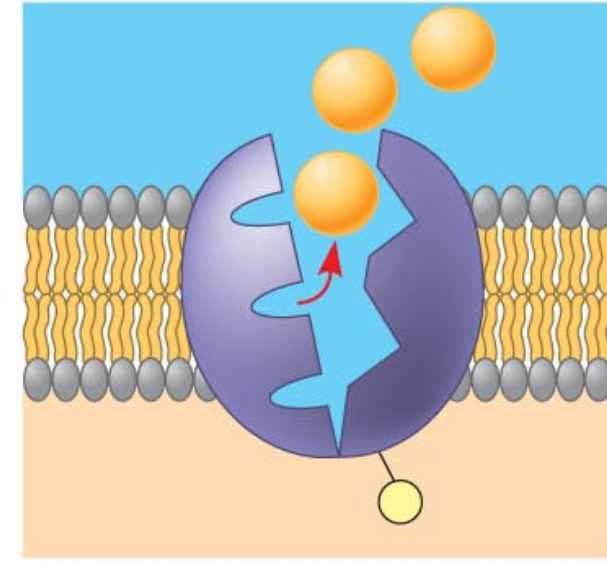




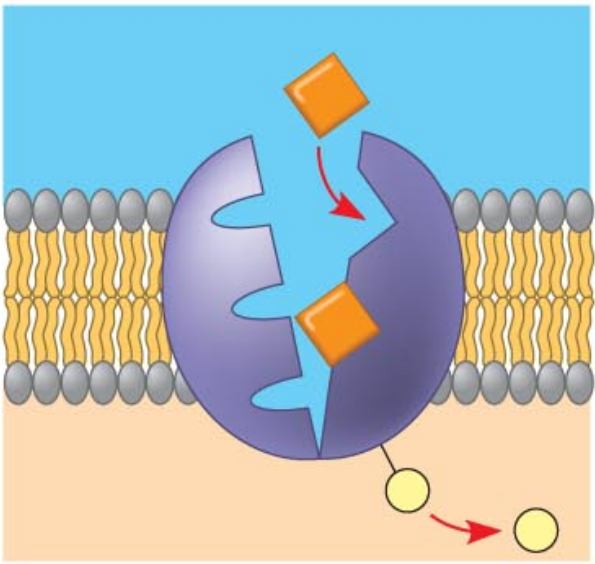
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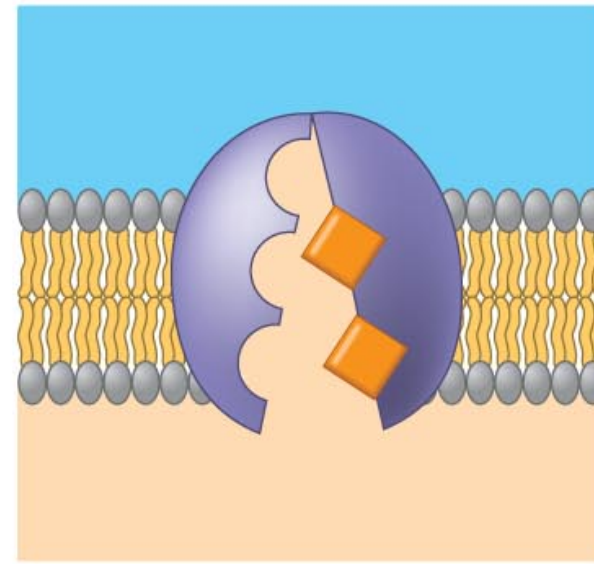
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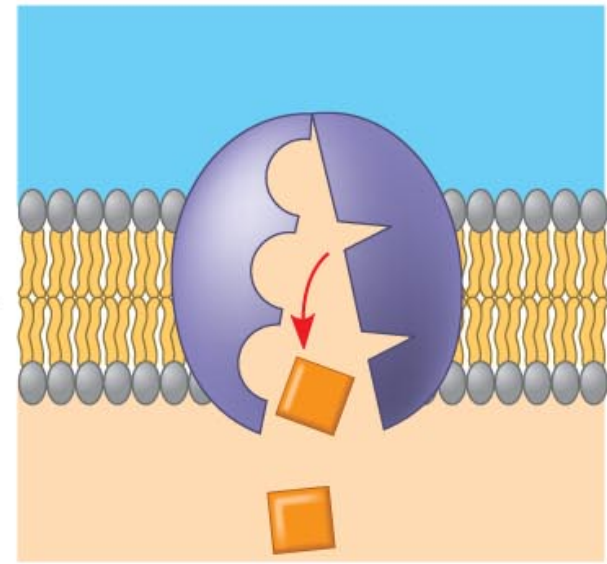
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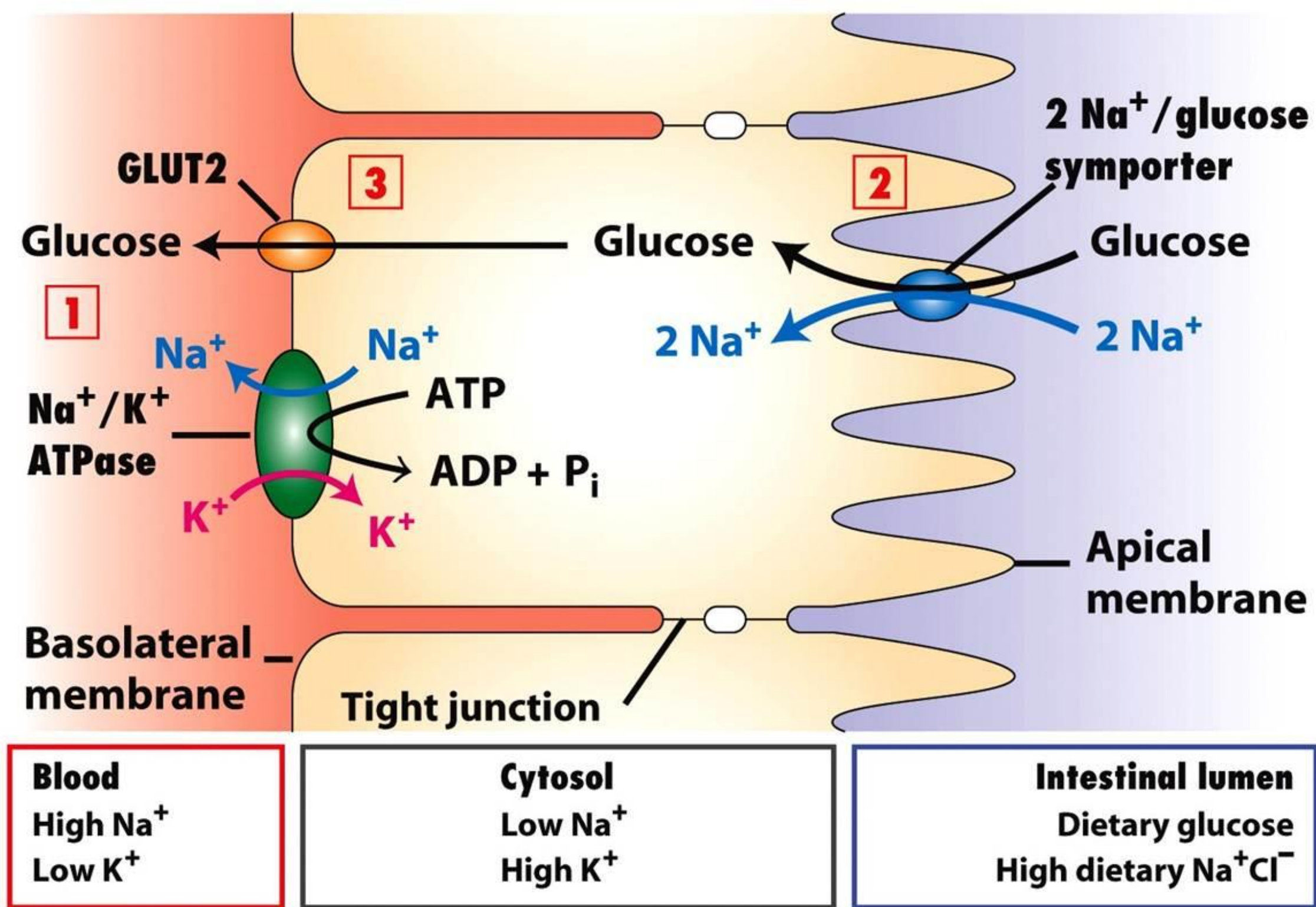


Figure 11-29
Molecular Cell Biology, Sixth Edition
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Sodium

- * Due to the relative abundance of sodium in the food supply, losses in sodium (functional hyponatremia) have more to do with physiological reasons than it does intake.
- * Conversely, elevation in sodium (hypernatremia) could be in part due to excess sodium intake.
- * *Determinations of plasma sodium levels detect changes in water balance rather than sodium balance.*
- * Largely hormonally regulated – aldosterone, ADH, atrial natriuretic peptide

Mild hyponatremia, hypernatremia and incident cardiovascular disease and mortality in older men: A population-based cohort study



S.G. Wannan

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^b Department of Popu

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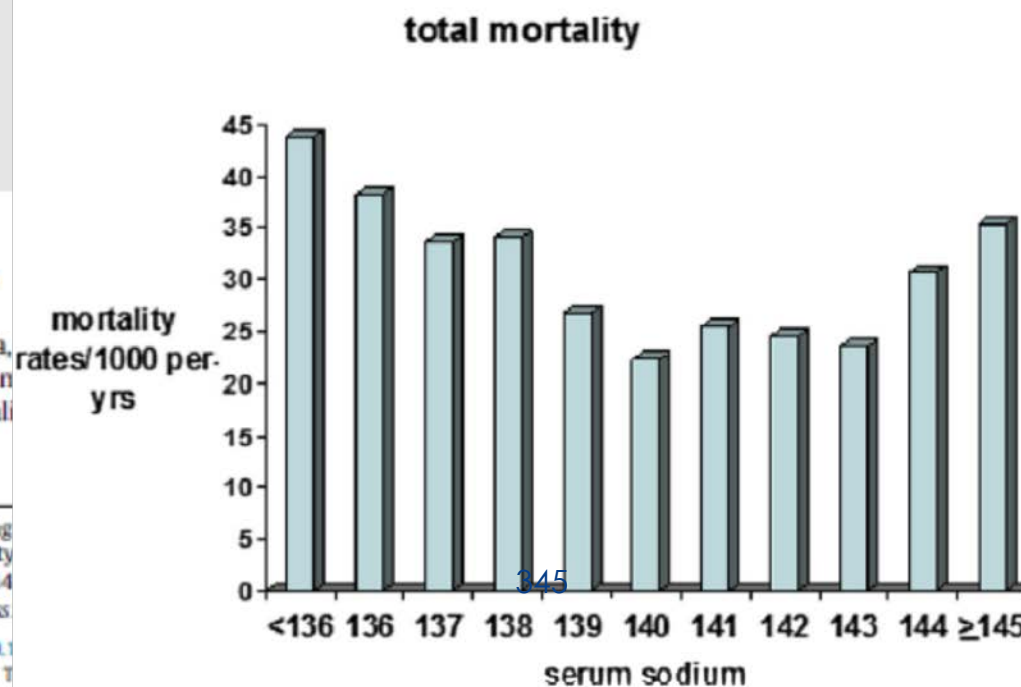
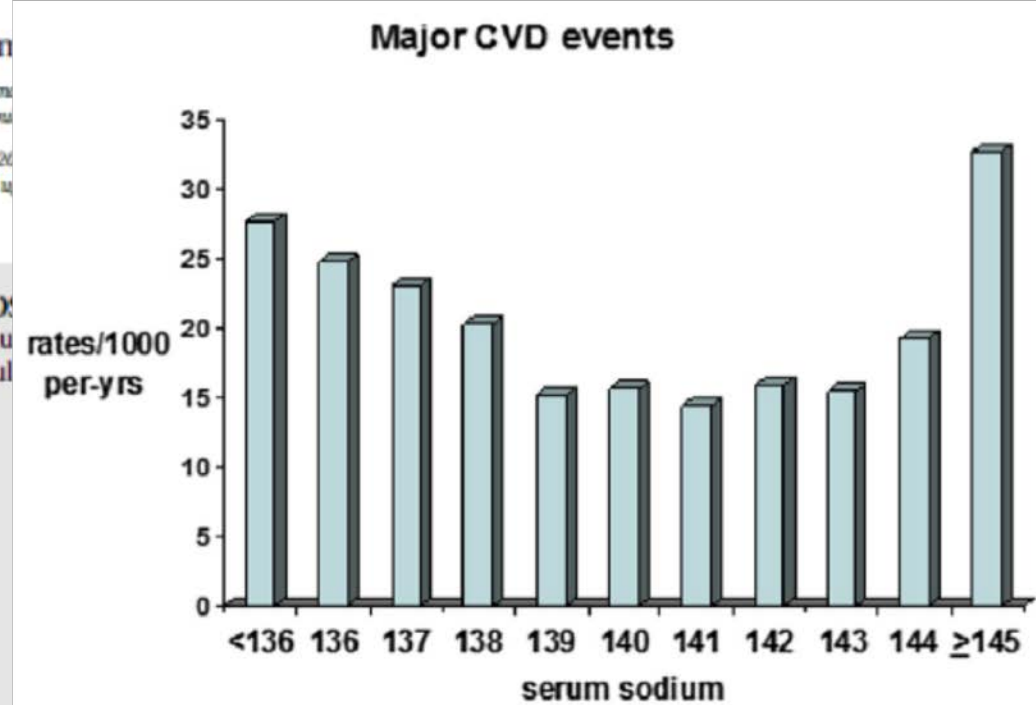
KEYWORDS:
Serum sodium
Cardiovascular
disease;
Stroke;
Mortality

Introduction

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Small Increases in Plasma Sodium Are Associated with Higher Risk of Mortality in a Healthy Population

Se Won Oh,¹ Seon Ha Jung Nam An,² Ho Su Sejoong Kim,^{2,4} Ki You Dong Wan Chae,^{2,4} Su and Ho Jun Chin^{2,4,5}

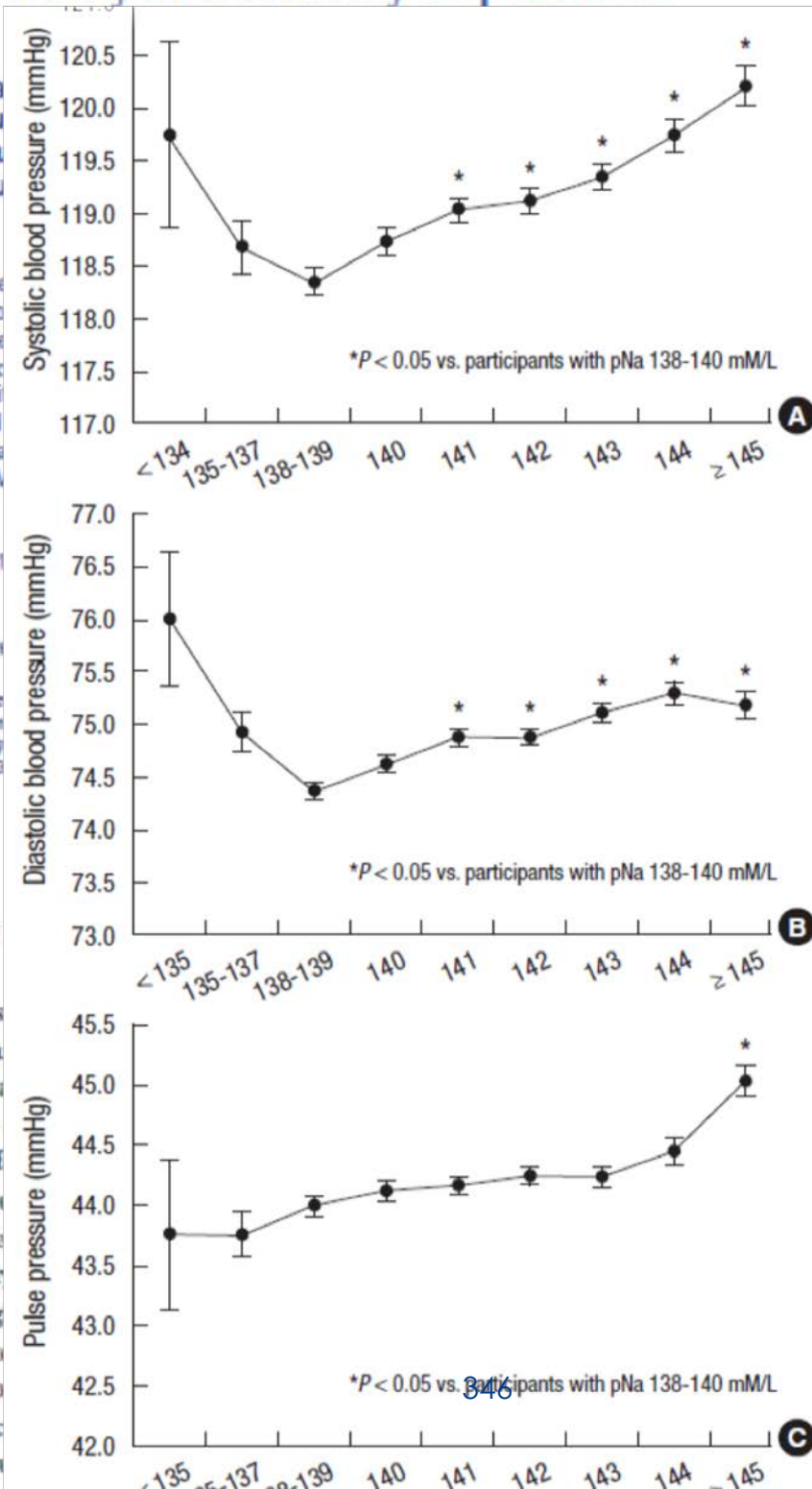
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INTRODUCTION

Elevated blood pressure is a major risk factor for cardiovascular disease (1). Much evidence suggests that salt intake plays an important role in the development of hypertension, and is associated with multiple factors that contribute to the pathogenesis of cardiovascular disease, including increased salt intake, and the excretion of sodium. Increased salt intake is associated with several compensatory mechanisms, including an increase in the plasma volume, which leads to an increase in the plasma volume, stimulation of the renin-angiotensin system, and increases in sympathetic activity. These compensations, a significant increase in blood pressure with progressive increases in salt intake, are associated with a higher risk of mortality in a healthy population.



of cardiovascular disease. Salt (pNa) is increased with progressive Na and BP had been reported a and BP, and estimated the risks of data collected from health included. Positive correlations (PP) were noted in participants aged ≥ 50 yr, SBP, DBP, its with metabolic syndrome. pNa were greater ($P < 0.001$). Increasing pNa in women aged 50 yr. In women, unadjusted risks for per adjustment, pNa ≥ 145 mM/L pNa and BP is stronger in older components. The incidence and in women aged ≥ 50 yr.

ium; Sex Characteristics

men pNa and SBP in middle-aged as known about the effect of pNa Na levels are reportedly related to 12, 13), and the incidence of mor- hyponatremia (13). Hyponatremia associated with medical conditions gnancy, and frequently result in er, the risk of mortality in subjects a has not been evaluated. n between pNa and BP stratified Korean population, and estimat- ity according to increases in pNa,

Sodium

Traditional Reference Range:

- * 135-148 mmol/l

Optimal Reference Range:

- * 139-142 mmol/L

Sodium

- * Symptoms of hypernatremia include dryness of mucous membranes, thirst, restlessness
- * Symptoms of hyponatremia include weakness, confusion, lethargy

Sodium – Elevated (functional hypernatremia)

Cause	Reason	Additional Inquiry
Dehydration	Sodium appears higher due to lower water content in plasma. Hydrating the client should correct this lab abnormality.	Other markers of dehydration including BUN, albumin, RBC, hemoglobin and hematocrit.
Hyperaldosterone	Aldosterone stimulates kidneys to reabsorb sodium back into the blood stream, and excrete potassium.	Elevated sodium, decreased potassium. Often will have high blood pressure. May have fatigue, headaches, muscle weakness.
High sodium intake		Diet journal

Elevated – Functional hypernatremia can be caused by an increase in sodium or a relatively loss in water only, making sodium appear higher than normal. Either one can cause movement of water out of cells and thus cellular dehydration.

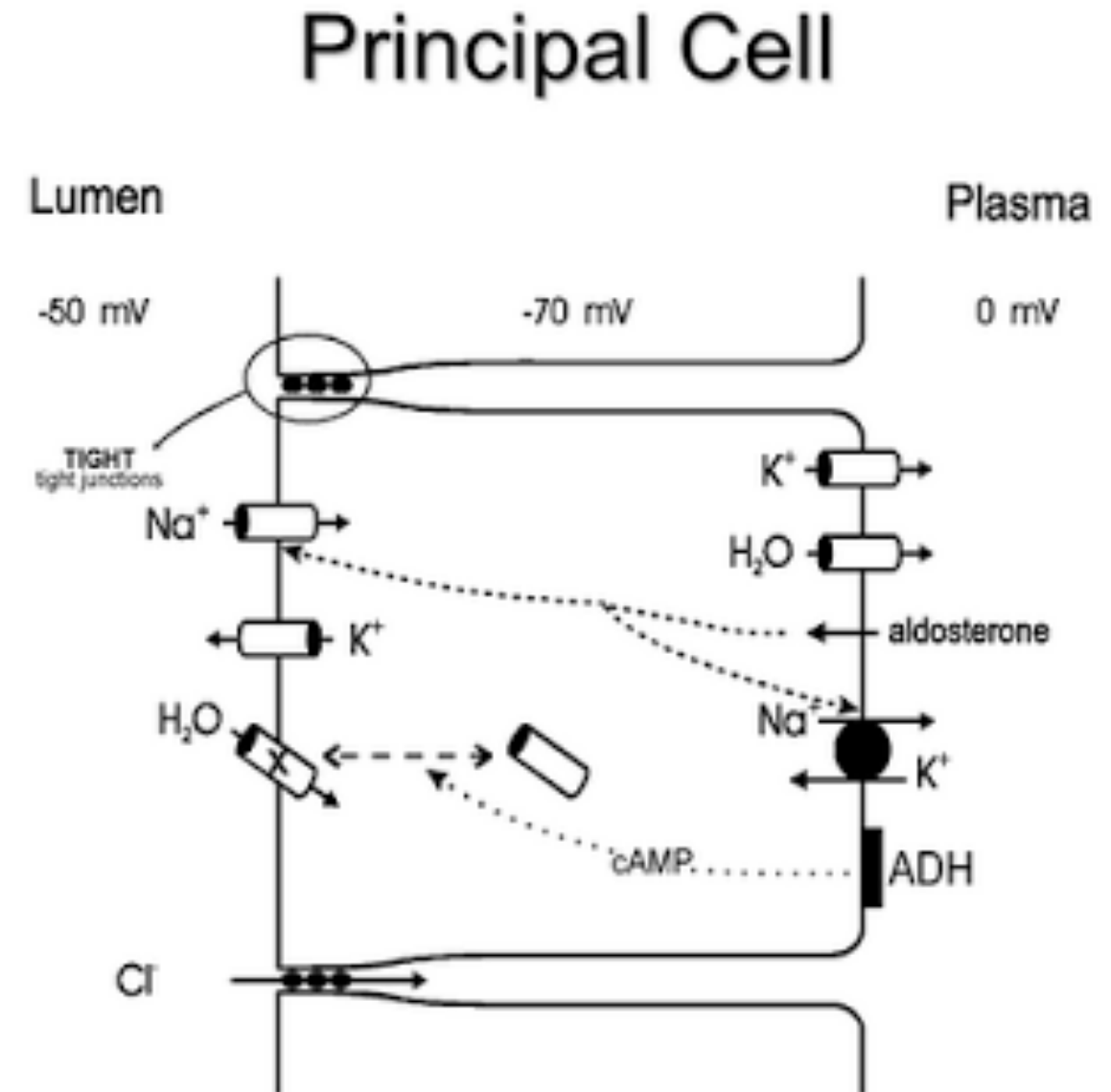
Elevated Sodium - Dehydration

- * Hemoconcentration
 - * Less water in plasma makes it appear there is more sodium per unit of water
 - * Will likely be true of a number of other markers as well (urea, electrolytes, RBC, hemoglobin, hematocrit)



Elevated Sodium – High Aldosterone

- * Aldosterone is released from the adrenal cortex
- * Acts on the principle cells of the nephron (kidney)
- * Results in the reabsorption of sodium (high in blood) and excretion of potassium (high in urine)



Sodium – Decreased (functional hyponatremia)

Decreased – Functional hyponatremia are either due to low sodium levels or excess water relative to sodium levels. This can lead to water movement out of extracellular fluid and into cells, thus causing cellular swelling.

Sodium – Decreased (functional hyponatremia)

Cause	Reason	Additional Inquiry
High serum glucose	Glucose causes a shift in osmolarity and tends to pull water out of cells and dilutes extracellular fluid, including sodium.	Evaluate glucose levels.
Hypocortisol secretion	Cortisol deficiency leads to an increase in CRH secretion, which acts as an ADH secretagogue. Cortisol also suppress ADH so if low, would allow for more secretion of ADH and thus hyponatremia.	Evaluate cortisol levels.
Glycosuria	The presence of an osmotically active and poorly reabsorbed solute in the can cause renal excretion of Na ⁺ and culminates in volume depletion	Evaluate urinalysis. If glucose is present, it may be contributing to low sodium.
Ketonuria	Hydroxybutyrate and acetoacetate (ketones) increase electrolyte loss in the urine, including sodium.	Evaluate urinalysis. If ketones are present, it may be contributing to low sodium.
Hypothyroidism	Mechanism unclear, though thought to be related to kidney function.	Evaluate thyroid markers.
Fluid loss (ie sweat)	If fluid loss accompanies excessive sodium loss, hyponatremia can result.	Inquire about excessive sweating, saltiness of sweat, or diarrhea.

High Serum Glucose

- * When glucose is elevated in the blood, it increases osmolarity of the blood and thus, water is pulled from cells in an attempt to dilute it and thus lower osmolarity
- * Adding water to the plasma can dilute the solutes, making sodium appear low

Low cortisol secretion

- * If cortisol is low, so to may CRH (corticotropin releasing hormone) from the hypothalamus.
- * CRH normally stimulates the posterior pituitary to release anti-diuretic hormone, which signals the kidneys to reabsorb more water
- * Cortisol also inhibits ADH thus, if cortisol is low, ADH may be elevated
- * Bottom line – if cortisol is low, there may be elevated ADH, which stimulates the kidneys to reabsorb water (not sodium) which can dilute the plasma and make it appear sodium is low

Sodium – Decreased (functional hyponatremia)

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Sodium – Interfering factors

Interfering Factors	
Elevated	Decreased
	High triglycerides or low protein
Medications - Anabolic steroids, corticosteroids, calcium, iron	Medications - Heparin, laxatives, diuretics

Potassium

- * Potassium is the most abundant intracellular cation and plays a number of important roles in the body.
- * In fact, it is estimated that 95-98% of the body's potassium is found within cells. Serum levels of potassium are relatively low and thus minor changes can be significant.

Potassium

- * Potassium has a number of important roles including helping to regulate acid-base balance, nerve conduction and action potentials, and muscle contraction, including the cardiac muscle.
- * The NaKATPase pump transports three sodium ions out of a cell while transporting two potassium ions inside the cell.
- * It is partly due to this discrepancy – three positively charged sodium ions to two positively charged potassium ions – that contributes to the *transmembrane potential* also known as the *resting membrane potential*.

Potassium

- * Potassium levels are influenced by intake, excretion, and distribution throughout the body.
- * Potassium can be impacted by hormones, including insulin and aldosterone, changes in acid-base balance, kidney function, and can be lost via skin or the gastrointestinal system.

Potassium

- * While potassium is necessary for basic cellular function of muscle and neuronal tissue, a number of studies have shown the lower ones serum potassium level, the higher the risk of cardiovascular risk and mortality.

The Relation of Serum Potassium Concentration with Cardiovascular Events and Mortality in Community-Living Individuals

Table 2. Associations between categories of serum potassium and all-cause mortality, CVD death, non-CVD death, and CVD events in MESA and CHS (pooled, $n=9651$)

Outcomes	Potassium Concentration, mEq/L				
	<3.5	3.5–3.9	4.0–4.4	4.5–4.9	≥5.0
All-cause mortality					
Events/P-Y	199/4086	723/18,835	1777/61,187	739/25,691	91/2758
Event rate (per 1000 P-Y)	48.70	38.39	29.04	28.76	32.99
Age, sex, race	1.33 (1.15 to 1.54)	1.28 (1.17 to 1.40)	1 (ref)	1.07 (0.98 to 1.16)	1.37 (1.11 to 1.69)
Fully adjusted ^a	1.13 (0.96 to 1.33)	1.18 (1.08 to 1.30)	1 (ref)	1.06 (0.97 to 1.16)	1.41 (1.12 to 1.76)
CVD death^b					
Events/P-Y	65/5858	233/24,635	549/75,102	212/31,245	28/3308
Event rate (per 1000 P-Y)	11.10	9.46	7.31	6.79	8.46
Age, sex, race	0.96 (0.74 to 1.24)	0.99 (0.85 to 1.16)	1 (ref)	1.26 (1.08 to 1.48)	1.60 (1.09 to 2.34)
Fully adjusted ^a	0.84 (0.63 to 1.12)	0.98 (0.79 to 1.09)	1 (ref)	1.21 (1.02 to 1.42)	1.50 (1.00 to 2.26)
Non-CVD death^b					
Events/P-Y	134/5858	490/24,635	1228/75,102	527/31,245	63/3308
Event rate (per 1000 P-Y)	22.87	19.89	16.35	16.87	19.04
Age, sex, race	0.89 (0.74 to 1.06)	0.91 (0.82 to 1.01)	1 (ref)	1.45 (1.31 to 1.61)	1.56 (1.21 to 2.02)
Fully adjusted ^a	0.99 (0.81 to 1.21)	1.02 (0.85 to 1.06)	1 (ref)	1.22 (1.10 to 1.36)	1.40 (1.07 to 1.83)
CVD events					
Events/P-Y	137/3300	472/16,590	1224/53,951	484/23,084	59/2417
Event rate (per 1000 P-Y)	41.52	28.45	22.69	20.97	23.17
Age, sex, race	1.58 (1.33 to 1.89)	1.24 (1.11 to 1.38)	1 (ref)	0.95 (0.85 to 1.05)	1.14 (0.88 to 1.49)
Fully adjusted ^a	1.13 (0.93 to 1.37)	1.08 (0.96 to 1.20)	1 (ref)	1.02 (0.91 to 1.13)	1.20 (0.90 to 1.56)

Data are displayed as Hazard Ratio (95% confidence intervals) for age, sex, race, and fully adjusted models. CVD, cardiovascular disease; MESA, Multi-Ethnic Study of Atherosclerosis; CHS, Cardiovascular Health Study; P-Y, person-years; ref, reference.

^aAdjusted for age, sex, race, study cohort, time-varying eGFR, diabetes mellitus, systolic BP, current smoking, pack-years smoking, ever having cancer, angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers, potassium-sparing diuretics, other diuretics, nonsteroidal anti-inflammatory drugs, potassium supplements, other antihypertensive medications, β blockers, and β agonists.

^bAnalyses and events per P-Y accounted for participants up to December of 2011.

Potassium

Traditional Reference Range

- * 3.5-5.5 mmol/L

Optimal Reference Range

- * 4.0-4.4 mmol/L

Potassium – Elevated (functional hyperkalemia)

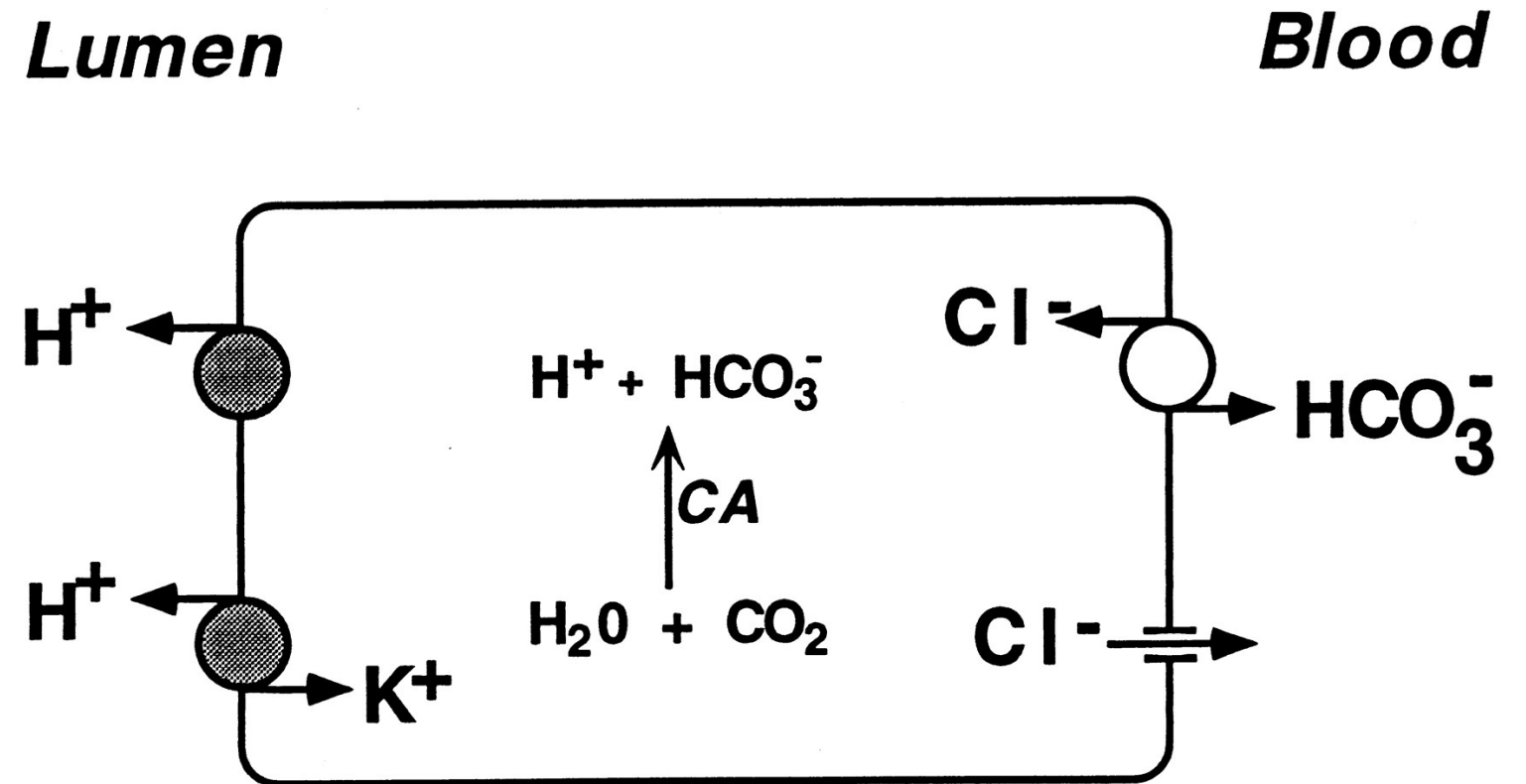
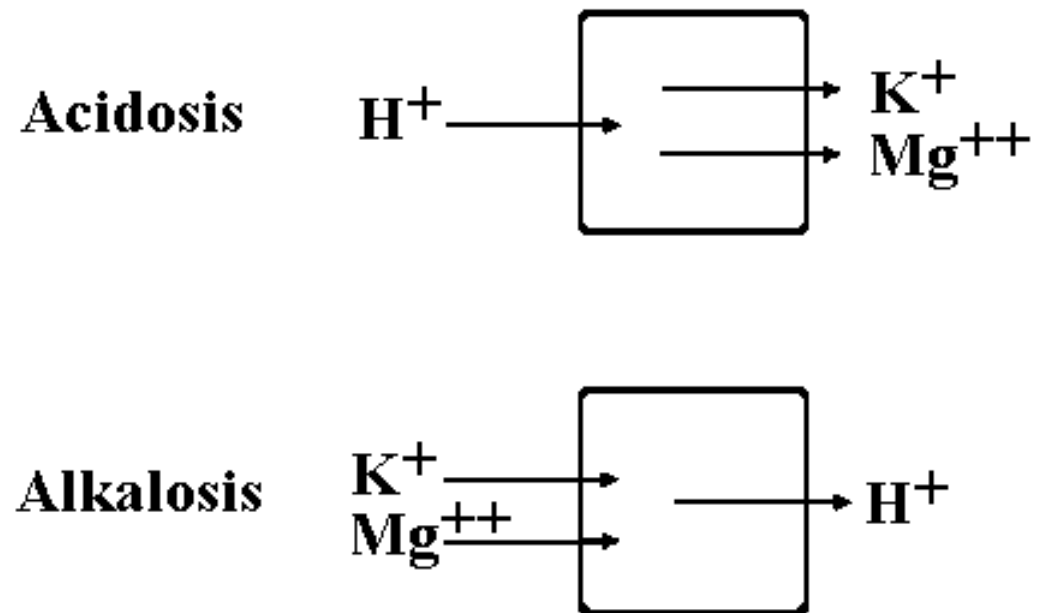
- * Functional hyperkalemia is generally due to poor excretion of potassium, or intracellular loss. High potassium intake could possibly cause an increase in serum potassium levels, though the body will likely regulate this. However, the kidneys can take up to three weeks to adapt to high potassium levels, so newly increased intake can be elevated on a blood chemistry for a few weeks before the body can compensate.

Potassium – Elevated (functional hyperkalemia)

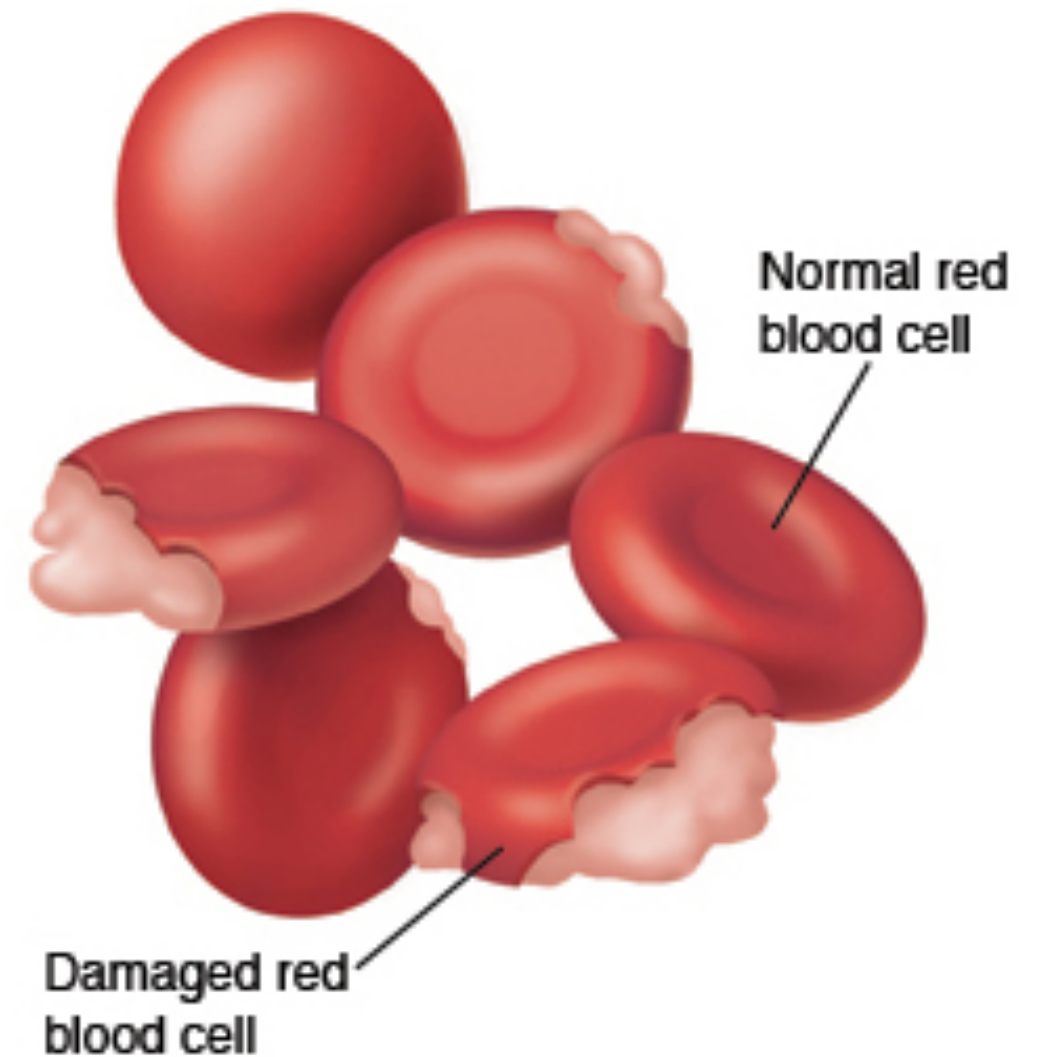
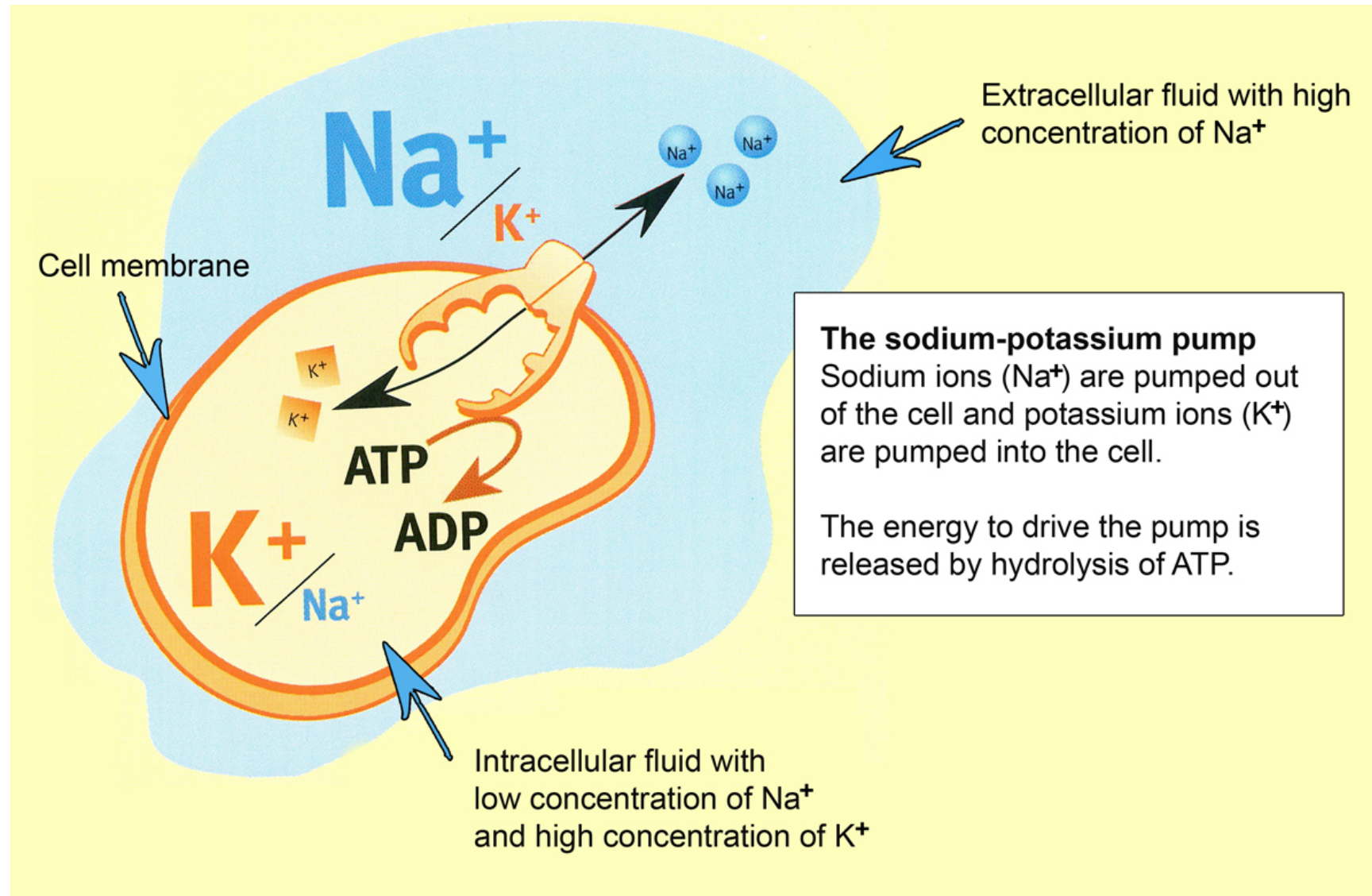
Cause	Reason	Additional Inquiry
Renal failure	The kidneys are the primary route of elimination thus, if they are not functioning properly, serum potassium levels will rise.	Evaluate other kidney-related marker including BUN, creatinine and a urinalysis.
Hypoaldosterone	Aldosterone produced by the adrenal glands will excrete sodium and reabsorb potassium. Thus if aldosterone is low, sodium will be excreted and potassium will remain in the body.	Consider evaluating adrenal hormone.
Functional acidosis	As the pH of the blood decreases (becomes more acidic), hydrogen ions will enter into cells and be exchanged with potassium ions, which will then increase in plasma.	Evaluate CO2 levels.
Hemolysis	Since potassium is found inside cells, if there is excessive cellular breakdown (Eg red blood cells), potassium will 'spill out' causing increases in plasma potassium.	Evaluate CBC panel for possible hemolytic tendencies.
Low Insulin or possibly insulin resistance	Insulin helps transport potassium into cells. Thus if insulin is low, potassium may remain elevated in serum.	Evaluate c-peptide.
Hyperglycemia	Acute hyperglycemia can increase osmolarity of extracellular fluid and promote potassium to leave cells, thus increasing serum levels.	Evaluate glucose markers.
Exercise	Potassium is released from exercising muscles, though once exercise has ended, potassium often quickly returns to normal.	

Functional Acidosis

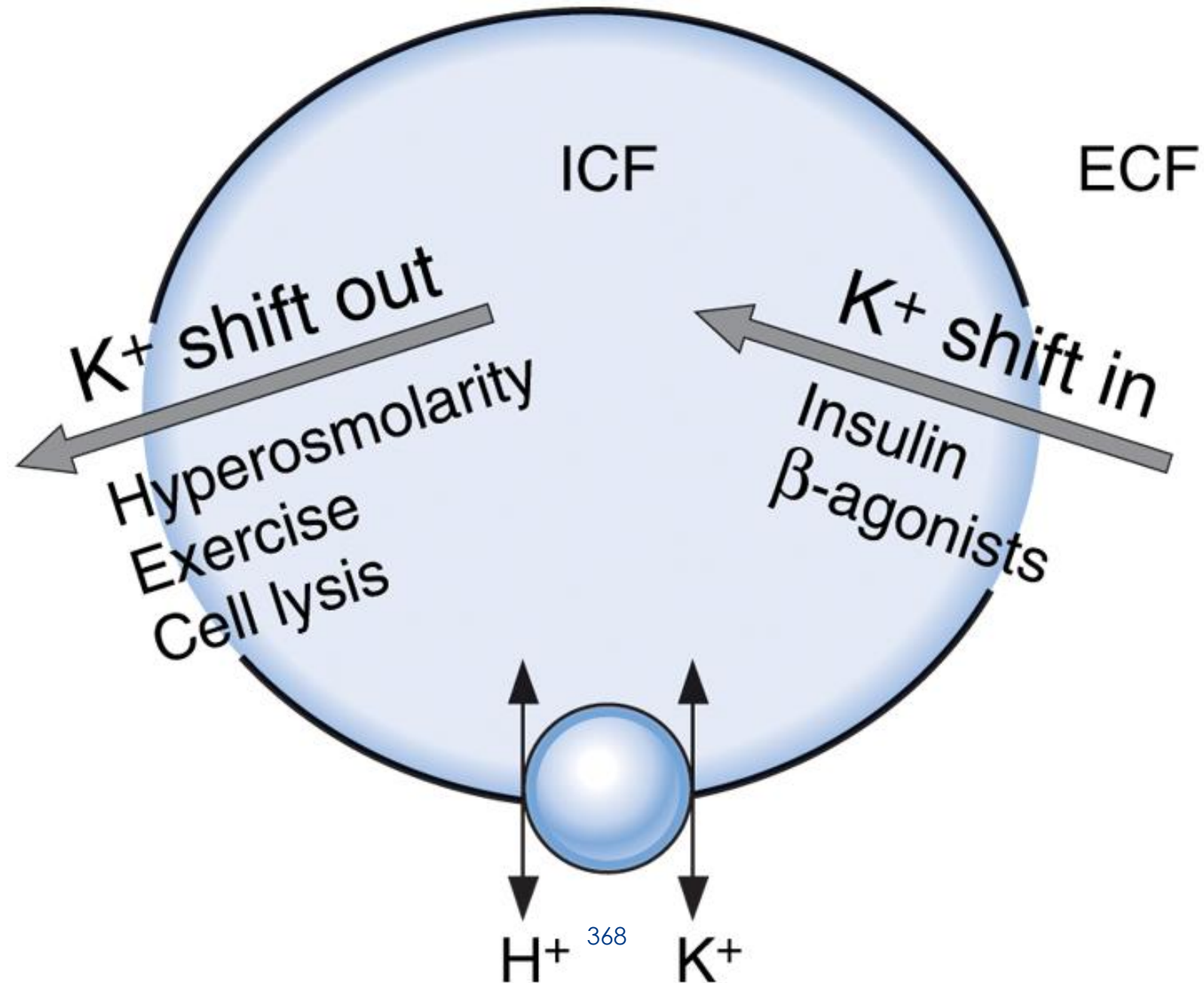
Table 7. Transcellular shifts at:



Hemolytic causes



Low Insulin



Potassium – Functional Hypokalemia

- * Functional hypokalemia exists with shifting potassium into cells, loss (eg GI tract), excess kidney excretion, or reduced intake.

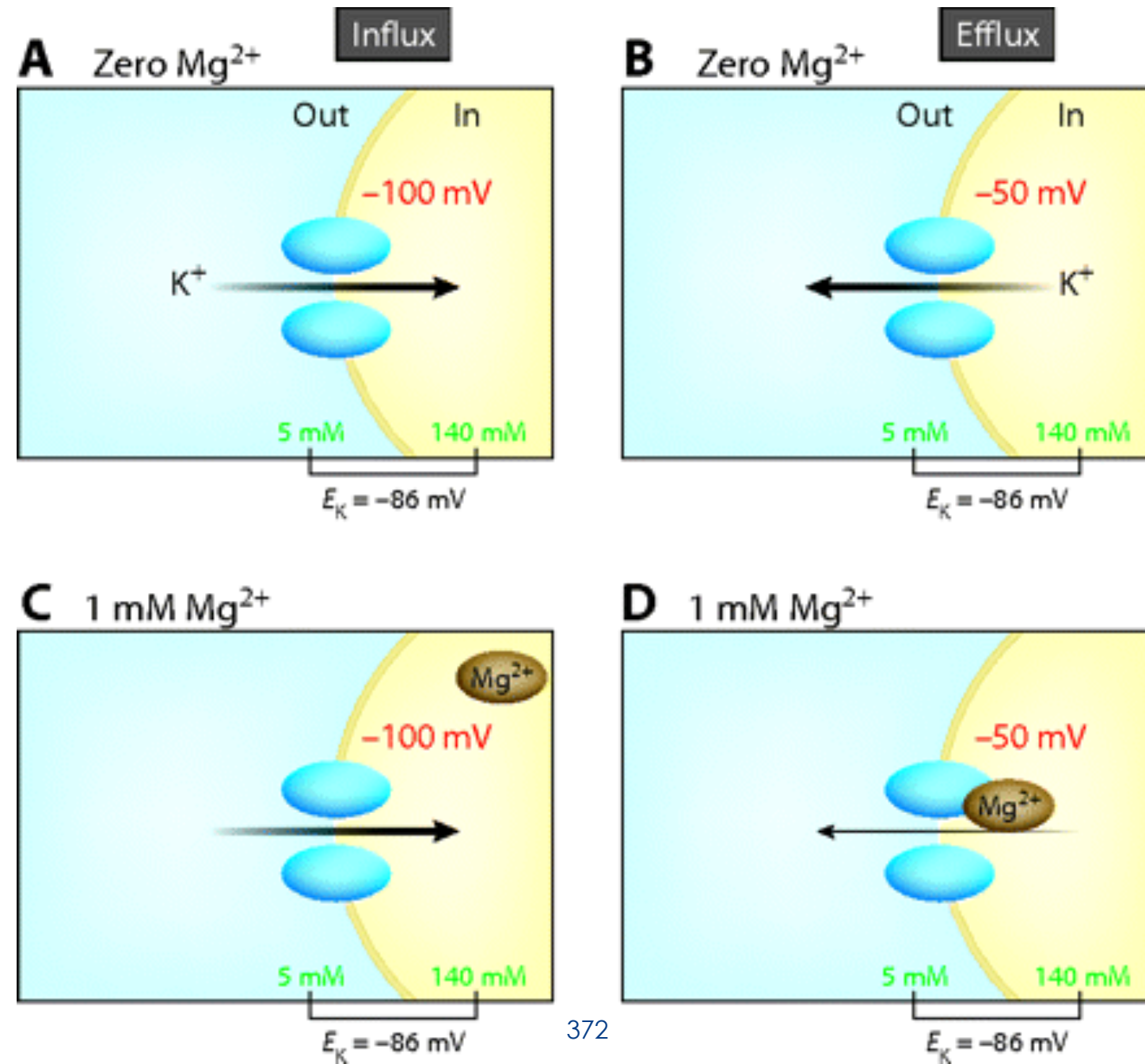
Potassium – Functional Hypokalemia

Cause	Reason	
Poor potassium intake		Evaluate diet history.
Alkalosis	When serum pH increases (more alkaline), hydrogen ions are released from cells into the blood stream in an attempt to acidify it, and potassium enters into cells to replace the hydrogen ions.	Evaluate CO2 levels and urine pH.
Hyperaldosterone	Excess aldosterone produced by the adrenal glands will excrete sodium and reabsorb potassium. Thus if aldosterone is elevated, sodium will be retained and potassium will be excreted.	Consider evaluating adrenal hormones.
Excessive fluid loss	Diarrhea and/or excessive perspiration can decrease potassium levels.	Client history.
Elevated insulin	Insulin helps transport potassium across cell membrane. Thus if elevated could lead to decreases in serum potassium.**	Evaluate c-peptide and other glucose markers.

Potassium – Functional Hypokalemia

Low magnesium	Intracellular magnesium is necessary for blocking potassium secretion into the urine in the kidney. Thus low potassium will not be corrected until magnesium deficiency is corrected.	Evaluate magnesium status.
Elevated estrogen	One study suggested estrogen not only lowers potassium, but potentiates aldosterone, thus enhancing this effect. Progesterone had no influence on potassium. Thus depending on when in a hormonal cycle a female is, as well as total circulating estrogen levels, may impact potassium levels.	Evaluate estrogen markers, including T3 uptake.
Increased catecholamine	Beta-adrenergic activity enhances potassium's entry into cells. Thus stress causing increased catecholamine release may potentially decrease potassium levels. This parallels the effect of other stress hormones cortisol and aldosterone.	

Low Magnesium



Potassium

Interfering Factors	
Elevated	Decreased
Opening and closing fist 10 times with a tourniquet results in potassium increase by 10-20%. (Tourniquet should be released after needle has entered vein.)	
Medications - Potassium-sparing diuretics, NSAIDS	
Excessive coagulation. Platelets release potassium during coagulation. (Rare.)	

Evaluating Sodium and Potassium

- * Sodium and potassium on a blood chemistry
- * High sodium, low potassium – possibly elevated aldosterone
- * Low sodium, high potassium – possibly decreased aldosterone
- * High sodium and potassium – aldosterone likely not involved; possibly dehydration
- * Low sodium and potassium – aldosterone likely not involved; possibly loss of electrolytes (sweating, diarrhea)

	High Aldosterone	Low Aldosterone	Dehydration	Electrolyte Loss
Sodium	↑	↓	↑	↓
Potassium	↓	↑	↑	↓

Carbon Dioxide

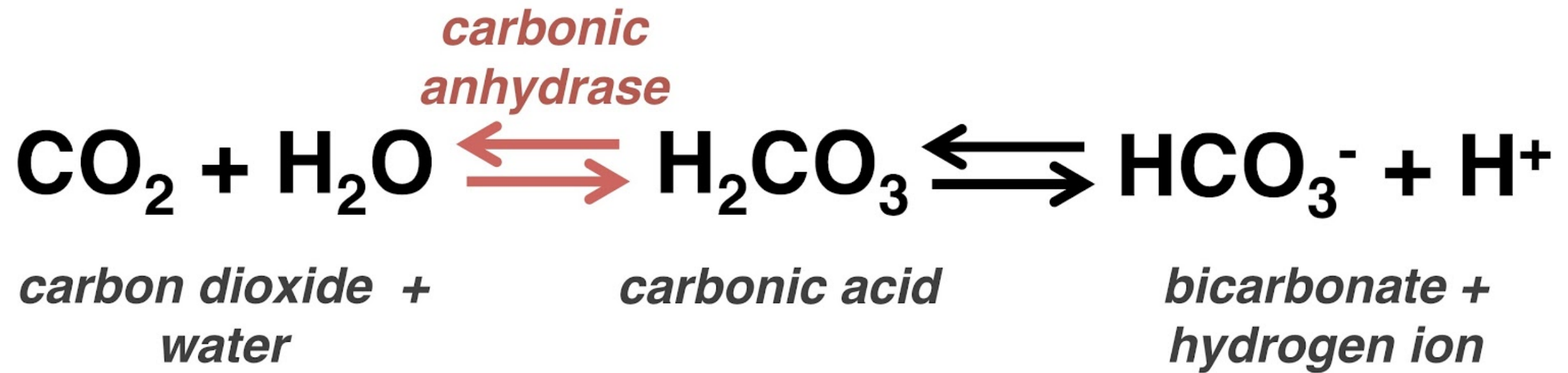
- * Serum carbon dioxide on a blood chemistry is not carbon dioxide the gas, but rather bicarbonate.
- * This test is a surrogate marker for carbon dioxide as a gas. (Arterial blood gasses measure the partial pressure of carbon dioxide, but are not routinely run outside of the clinical setting.)

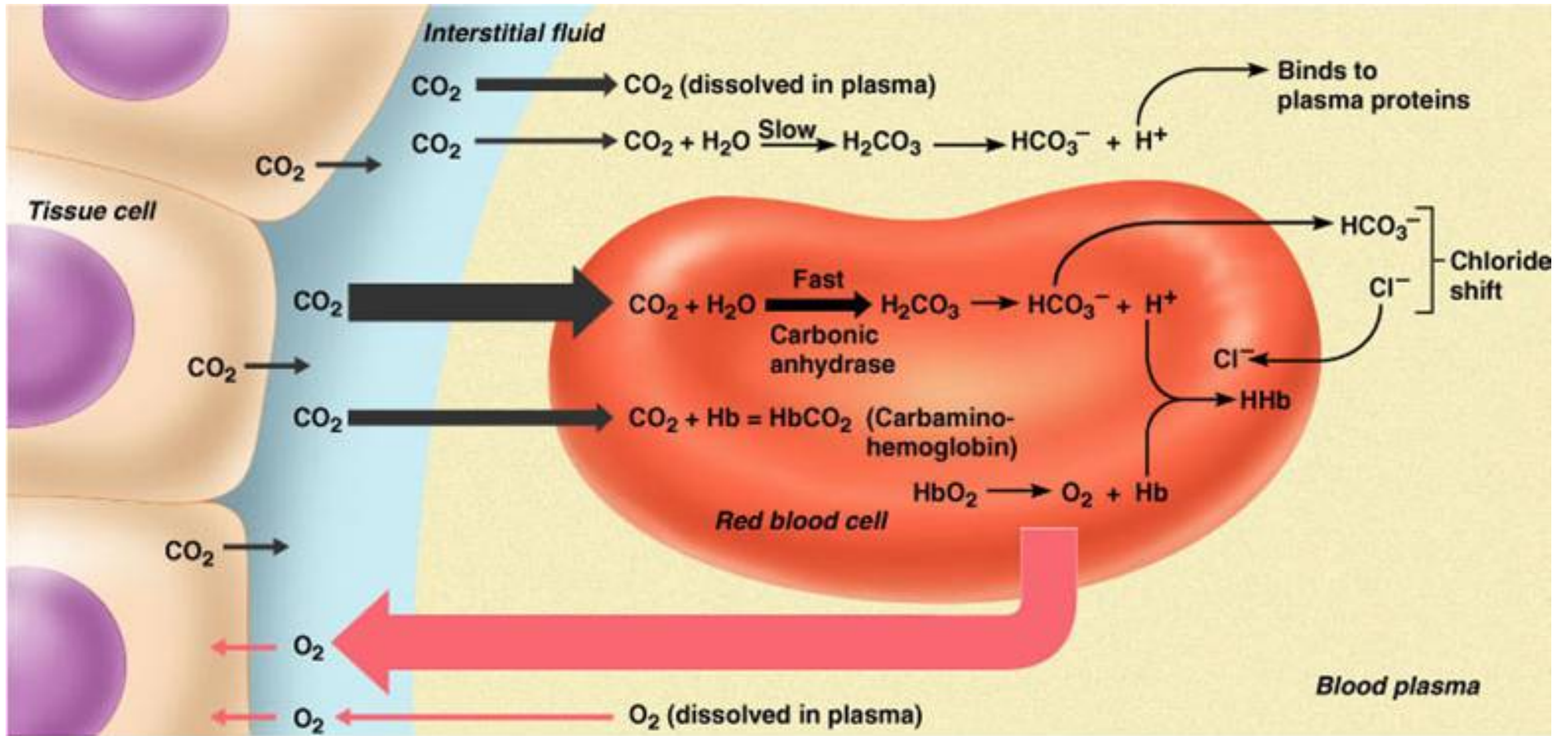
Carbon Dioxide

- * When carbon dioxide leaves cells as a byproduct of cellular respiration, when it enters blood it can quickly turn into hydrogen ions and thus significantly lower serum pH. This occurs via the carbonic anhydrase pathway, that states: $\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 \rightarrow \text{HCO}_3^- + \text{H}^+$ When hydrogen ions enter the blood stream, they can combine with bicarbonate (HCO_3^-) and be neutralized into CO_2 . Due to the excess CO_2 , respiration would increase to remove the elevated levels of CO_2 .

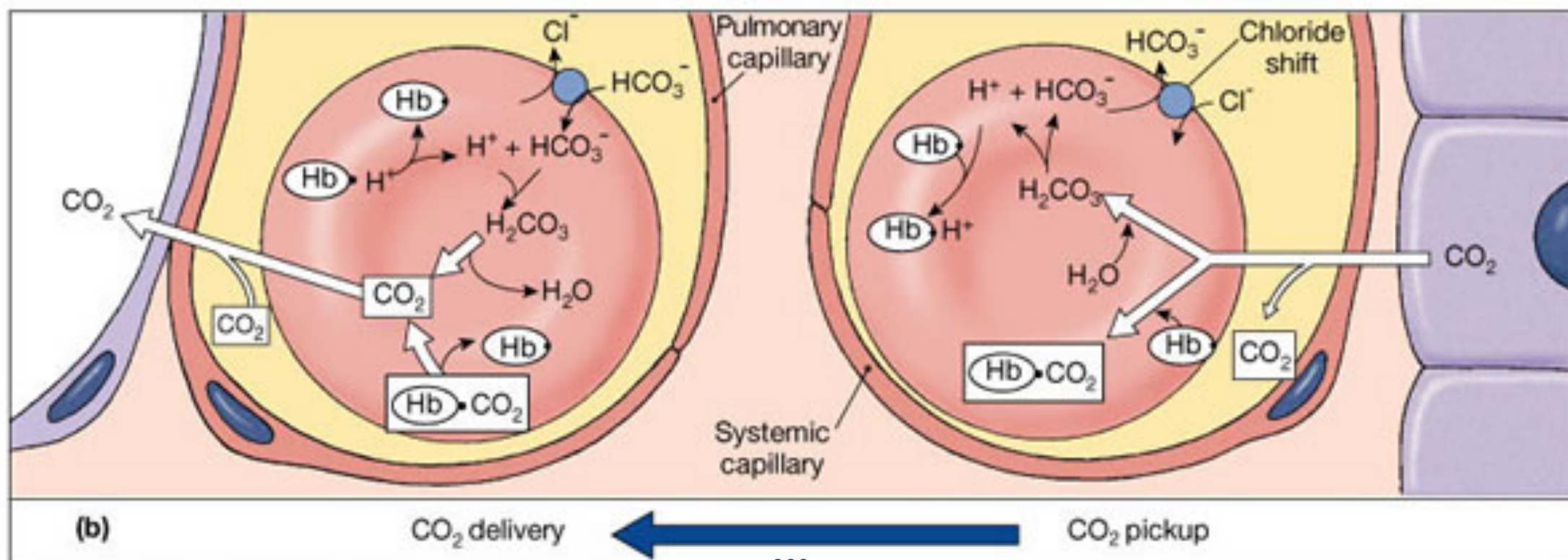
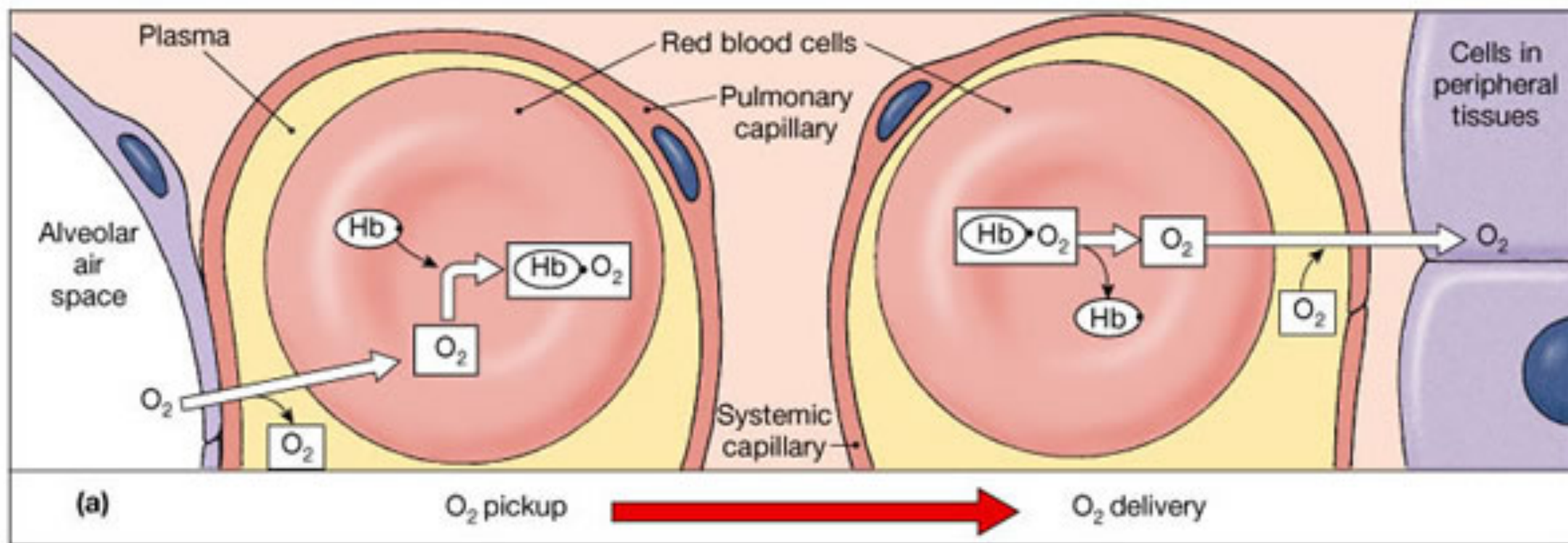
Carbon Dioxide

- * When CO₂ combines with water, hydrogen ions can be formed. Therefore CO₂ travels back to the lungs for exhalation as bicarbonate, hence the relationship between CO₂ and bicarbonate, and the clinical utility of this marker. And because of the chloride shift, bicarbonate and chloride levels are often inversely related.





(a) Oxygen release and carbon dioxide pickup at the tissues



Carbon Dioxide

- * Generally speaking the lower the CO₂ on a blood chemistry, the lower the pH of the blood (more acidic) and the higher the CO₂, the higher the pH (more alkaline). However this is not an accurate evaluation of pH. pH has more to do with the ratio of bicarbonate and the partial pressure of CO₂ (PCO₂) than it does with either individually. Therefore with only one of the values, pH is merely a loose estimation.
- * The kidneys are the primary organ regulating bicarbonate levels. They also excrete acids and for these two reasons, are a major regulators of acid-base balance in the body.

Serum anion gap, bicarbonate and biomarkers of inflammation in healthy individuals in a national survey

Wildon R. Farwell MD MPH, Eric N. Taylor MD MSc

Table 3: Adjusted differences in inflammatory biomarkers, by quartile of serum bicarbonate level

Biomarker	Quartile of serum bicarbonate level; difference in biomarker (95% CI)				<i>p</i> value for trend
	Quartile 4 (> 25 mmol/L)	Quartile 3 (25 mmol/L)	Quartile 2 (23–24 mmol/L)	Quartile 1 (< 23 mmol/L)	
Leukocyte count, × 10 ⁹ /L					
Adjusted for age	Referent	0.3 (0.0 to 0.4)	0.7 (0.3 to 1.0)	1.0 (0.7 to 1.3)	< 0.01
Adjusted for multiple variables*	Referent	0.1 (–0.0 to 0.3)	0.4 (0.2 to 0.6)	0.7 (0.3 to 1.0)	< 0.01
C-reactive protein level, nmol/L					
Adjusted for age	Referent	5.6 (1.2 to 10.1)	8.2 (5.5 to 11.0)	15.4 (11.4 to 19.3)	< 0.01
Adjusted for multiple variables*	Referent	1.6 (–2.7 to 6.0)	2.1 (–1.0 to 5.1)	4.0 (0.9 to 7.2)	0.02

Note: CI = confidence interval.

*Adjusted for age, sex, body mass index, ethnic background, use of alcohol, serum levels of cotinine, albumin, creatinine, uric acid and urea, and dietary protein intake.

inflammatory biomarkers in a healthy sample of the general population. Further studies are needed to elucidate the relation between acid–base status and inflammation.

Higher levels of inflammatory biomarkers are associated with increased mortality and the development of chronic disease. A recent study reported that all-cause mortality increased 11% for every increase of $1.0 \times 10^9/L$ in leukocyte count above $3.5 \times 10^9/L$.¹ Other studies also reported a significant relation between mortality and

Study population

We obtained data for individuals who participated in the National Health and Nutrition Examination Survey in 1999/2000, 2001/02, 2003/04 or 2005/06. The design of the survey has been described previously.¹² In brief, the survey provides nationally representative cross-sectional data on the health status of the general population of the United States. Participants

From the Massachusetts Veterans Epidemiology Research and Information Center, VA Boston Healthcare System, and the Division of Aging, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School (Farwell);

Plasma bicarbonate and risk of type 2 diabetes mellitus

Table 3: Odds ratios of type 2 diabetes mellitus during the 10-year follow-up period, by baseline level of plasma bicarbonate

Group or model	Quartile (plasma bicarbonate level, mmol/L); OR (95% CI)*				<i>p</i> for linear trend
	Quartile 1 (< 20.6)	Quartile 2 (20.6–22.3)	Quartile 3 (22.4–23.9)	Quartile 4 (> 23.9)	
Cases, no.	188	165	139	138	
Controls, no.	189	176	183	182	
Model					
1†	Referent	0.93 (0.69–1.25)	0.75 (0.55–1.01)	0.74 (0.55–1.01)	0.03
2‡	Referent	0.92 (0.67–1.25)	0.70 (0.51–0.97)	0.75 (0.54–1.05)	0.04

Note: CI = confidence interval, OR = odds ratio.

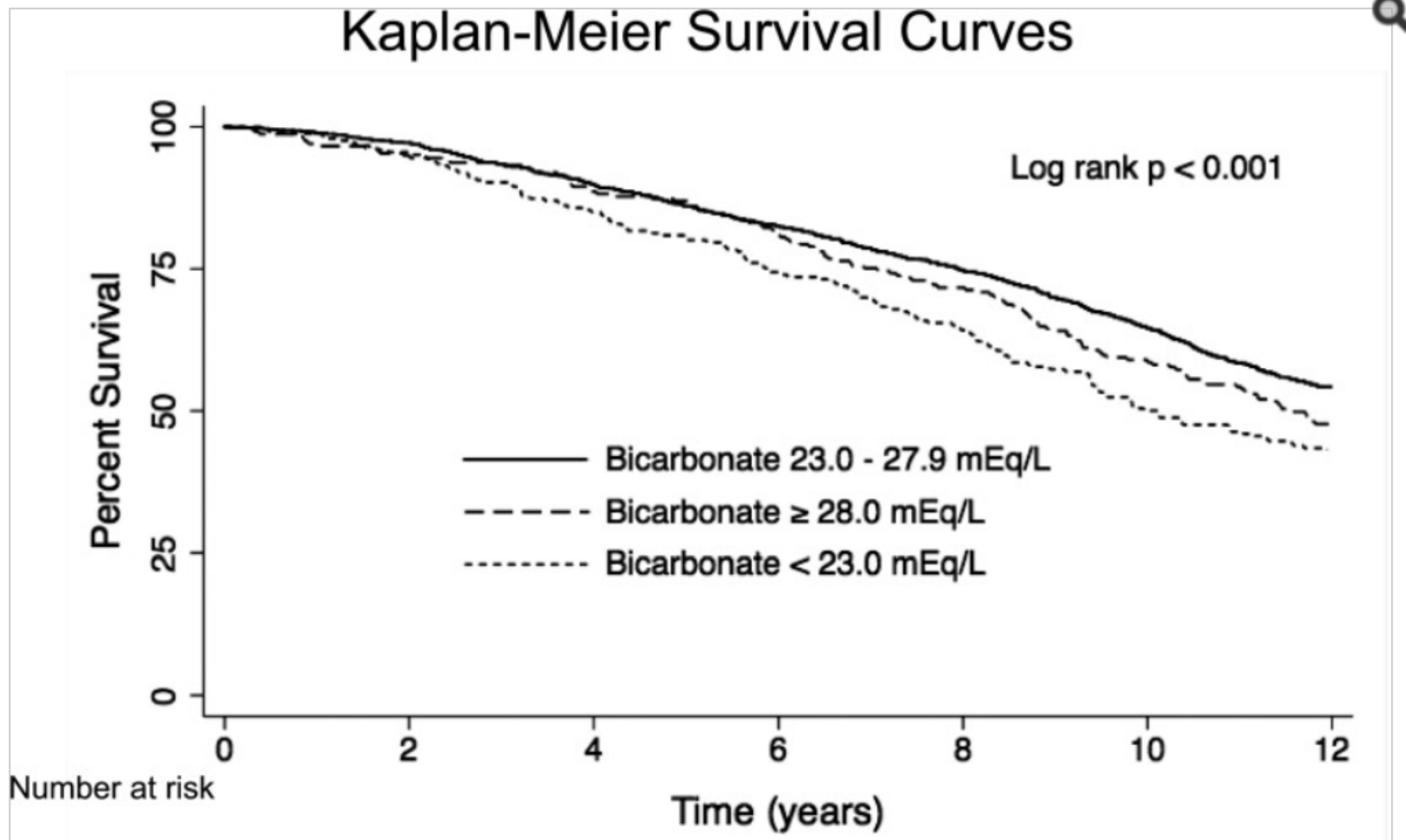
*Unless otherwise stated.

†Adjusted for matching factors: age at blood draw (continuous), ethnic background (white v. non-white) and fasting status at blood draw (binary). *n* = 630 cases and 730 controls.

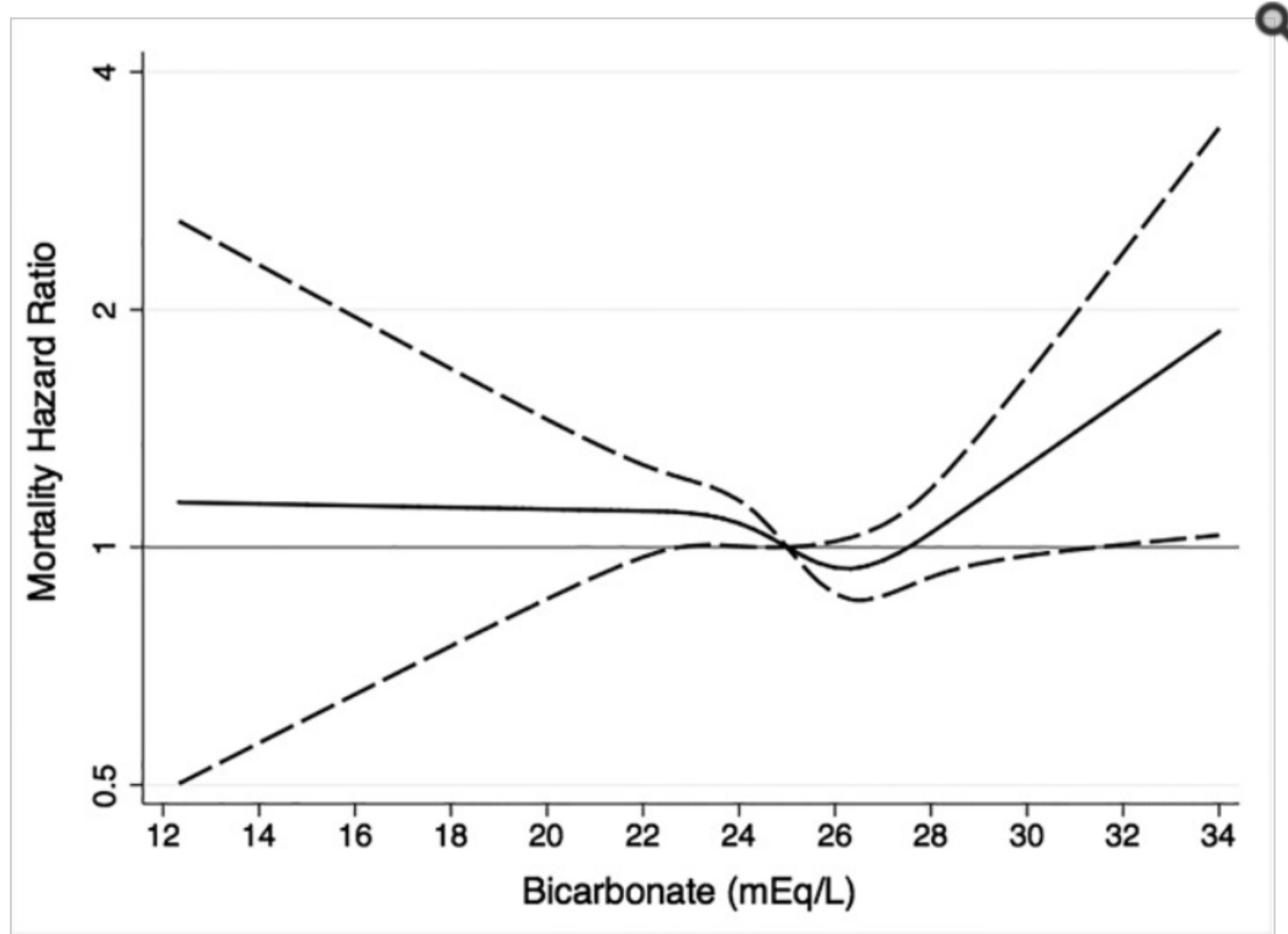
‡Adjusted for matching factors, body mass index (continuous), history of hypertension (binary) and plasma creatinine (< 0.6 mg/dL, 0.6–0.8 mg/dL, > 0.8 mg/dL). *n* = 629 cases and 729 controls. One case and one control in this model were missing data for body mass index.

the administration of ammonium chloride results in reduced tissue insulin sensitivity.³ Subsequent studies in rat models have suggested that metabolic acidosis decreases the binding of insulin to its receptors.^{4,5} Finally, metabolic acidosis may also increase cortisol production,⁶ which in turn is implicated in the development of insulin resistance.⁷

men.¹⁰ Other biomarkers associated with metabolic acidosis, including higher levels of serum ketones,¹¹ lower urinary citrate excretion¹² and low urine pH,¹³ have been associated in cross-sectional studies with either insulin resistance or the prevalence of type 2 diabetes mellitus. However, it is unclear whether these associations are a cause or consequence. We sought to address



- * Raphael, Kalani L., Rachel A. Murphy, Michael G. Shlipak, Suzanne Satterfield, Hunter K. Huston, Anthony Sebastian, Deborah E. Sellmeyer, et al. 2016. "Bicarbonate Concentration, Acid-Base Status, and Mortality in the Health, Aging, and Body Composition Study." *Clinical Journal of the American Society of Nephrology*: CJASN 11 (2): 308–16. doi:10.2215/CJN.06200615.



- * Raphael, Kalani L., Rachel A. Murphy, Michael G. Shlipak, Suzanne Satterfield, Hunter K. Huston, Anthony Sebastian, Deborah E. Sellmeyer, et al. 2016. "Bicarbonate Concentration, Acid-Base Status, and Mortality in the Health, Aging, and Body Composition Study." *Clinical Journal of the American Society of Nephrology* : CJASN 11 (2):308–16. doi:10.2215/CJN.06200615.

Association of Serum Bicarbonate Levels With Gait Speed and Quadriceps Strength in Older Adults

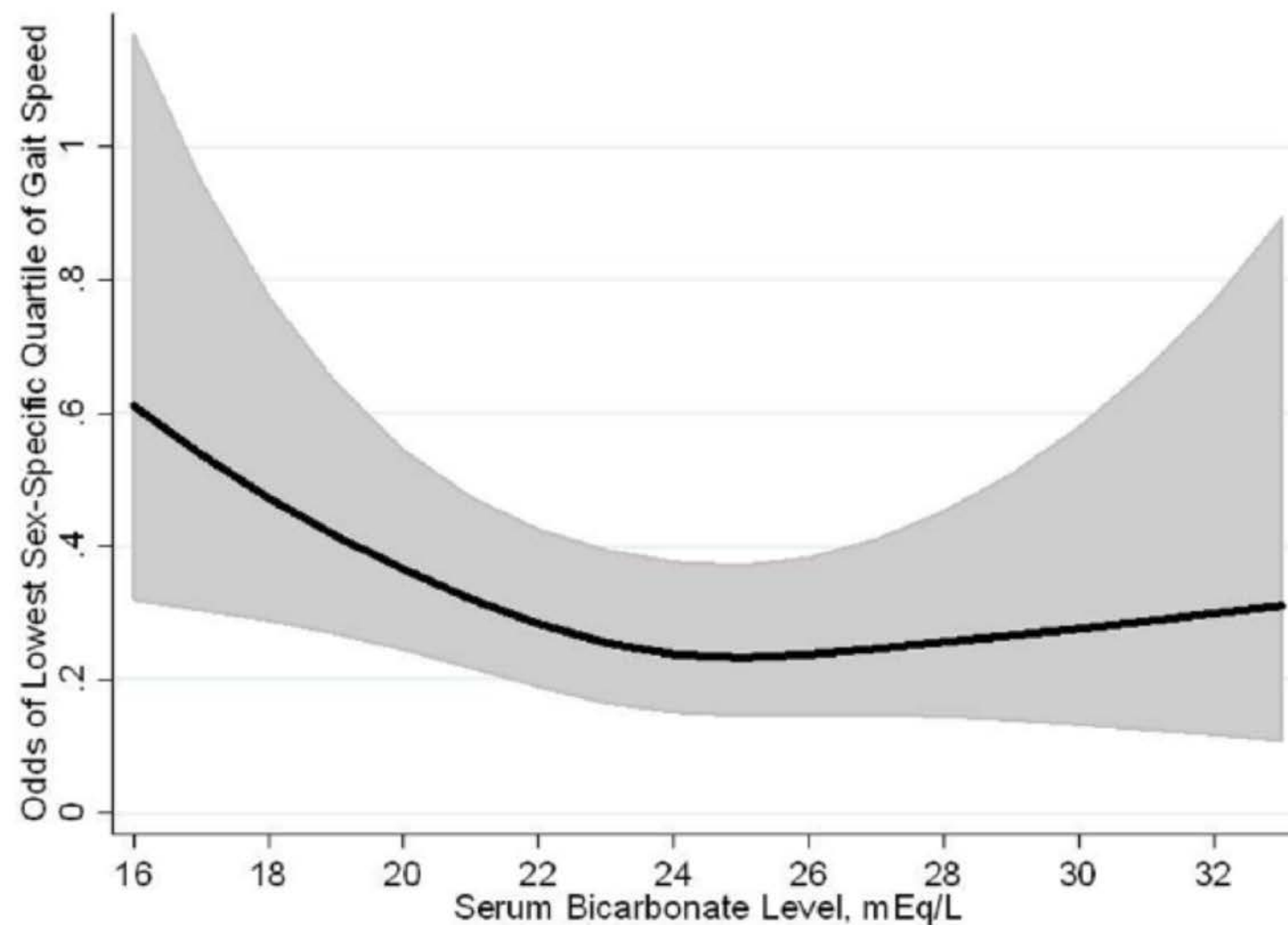


Figure 1. Restricted cubic spline showing the fully adjusted association of serum bicarbonate level with odds of low gait speed in 2,675 participants of the National Health and Nutrition Examination Survey 1999 – 2002.

Carbon Dioxide

Traditional Reference range:

- * 20-32 mg/dL or 20-32 mmol/L

Optimal Reference Range:

- * 25-28 mg/dL or 25-28 mmol/L

Carbon Dioxide - Elevated

Elevated – Functionally elevated CO₂ levels on a blood chemistry generally suggests excess alkalinity, though compensated chronic respiratory acidosis can also elevate CO₂ levels. The latter is often caused by chronic hypoventilation, poor gas exchange across the lungs, low potassium or low phosphorus.

Carbon Dioxide - Elevated

Cause	Reason	Additional Inquiry
Acute vomiting	Loss of hydrogen ions in the form of hydrochloric acid, causes a relative alkalosis and thus elevated CO ₂ levels.	Client history.
Functional metabolic alkalosis	Causes can include vomiting, low potassium (excess renal ammonia excretion), hyperadrenal function (elevated aldosterone leads to loss of potassium and hydrogen ions in urine), or certain medications.	Further evaluate potassium, sodium, etc.
Chronic respiratory acidosis (hypoventilation)	If there is impaired gas exchange in the lungs (eg asthma, emphysema) CO ₂ (gas) will tend to build up in the blood stream causing excess hydrogen ion formation and an increase in bicarbonate to compensate. 389	Evaluate respiration rate. If low (below 13 breaths per minute), this may be the cause of elevated CO ₂ on a blood chemistry.

Carbon Dioxide – Decreased

Decreased – Functionally decreased CO₂ levels on a blood chemistry generally suggests excess acidity, though compensated chronic respiratory alkalosis can also decrease CO₂ levels. The latter is often caused by chronic hyperventilation.

Carbon Dioxide – Decreased

Cause	Reason	
Metabolic acidosis	Excess hydrogen ions from acids (ketones, lactic acid, organic acids) are combined with bicarbonate, thus lowering CO ₂ level on a blood chemistry. Could also be a thiamin deficiency.	
	Hyperchloremia	
Chronic respiratory alkalosis (hyperventilation)	Hyperventilation decreases CO ₂ (gas) levels, which lowers hydrogen ion content and thus increases the pH (alkalosis). Due to the alkaline state, bicarbonate is excreted by the kidneys, leading to low CO ₂ on a blood chemistry.	Evaluate respiration rate. A respiratory rate above 18 breaths per minute may be contributing to low CO ₂ levels on a blood chemistry.

Dietary Acid, Age, and Serum Bicarbonate Levels among Adults in the United States

Afolarin Amodu* and Matthew K. Abramowitz*

Greater dietary acid has been associated with lower serum bicarbonate levels in patients with CKD. Whether this association extends to the general population and if it is modified by age are unknown.

Participants in the highest quartile of net endogenous acid production had 0.40 mEq/L lower serum bicarbonate and a 33% higher likelihood of acidosis compared with those participants in the lowest quartile.

Greater dietary acid is associated with lower serum bicarbonate in the general US population, and the magnitude of this association is greater among middle-aged and elderly persons than younger adults.

NEAP (mEq/d)=[54.5×protein (g/d)/potassium (mEq/d)]−10.2

individuals without kidney disease (4). This subclinical acidosis, mediated in part by the effects of aging and the Western diet, may have important sequelae on bone, skeletal muscle, and insulin resistance (5–7). Oral alkali administered to postmenopausal women has reduced bone resorption, improved bone mineral density, and decreased urinary nitrogen excretion, suggesting that acidosis has clinical sequelae even in this population (8–10).

In normal subjects, endogenous acid production, which

acidosis is associated with adverse outcomes on a population-wide level, even in people without kidney disease (6,7,13–15), determining the extent to which dietary acid may be a modifiable contributor to low-grade acidosis could have important public health implications. In addition, because older persons have lower capacity to excrete an acid load (16), this effect might be modified by age.

We hypothesized that dietary acid would be associated with acid-base status in the general population

Chloride

- * Chloride generally moves past cell membranes to counteract the negative charge of bicarbonate.
- * Specifically, during cellular respiration, as CO_2 and hydrogen ions (H^+) increase, bicarbonate (HCO_3^-) enters the cell to act as a buffer. As bicarbonate enters, chloride leaves (eg chloride shift) to maintain electrical neutrality across cell membranes.
- * Shifts in chloride on a blood chemistry do not necessarily reflect total body levels, but rather compensatory mechanisms for other physiological events, such as acid-base disturbances.

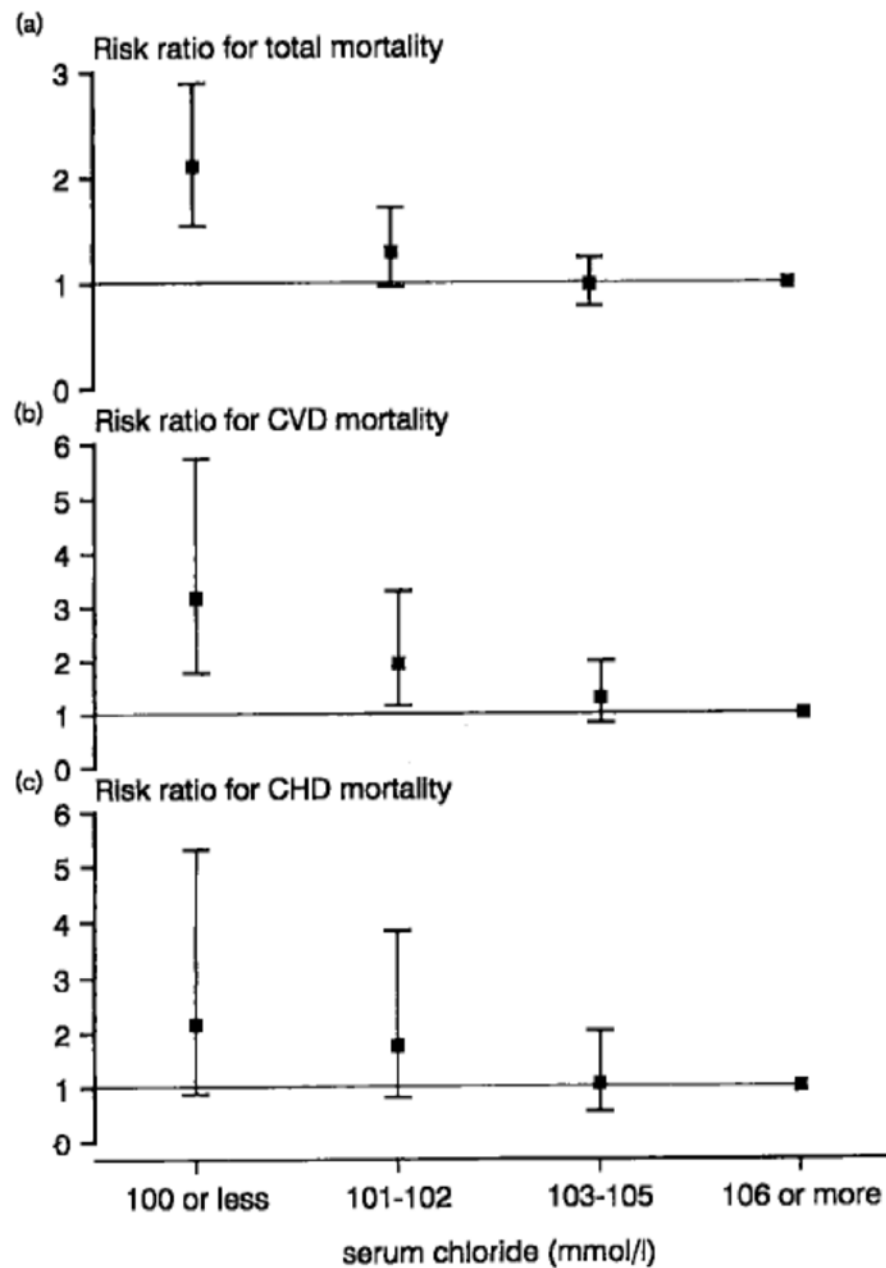
Chloride

- * Chloride has a number of important roles in addition to being an electrolyte.
- * As previously mentioned, chloride is secreted with protons from parietal cells to make hydrochloric acid in the stomach. Phagocytic white blood cells use chloride to help neutralize foreign substances (eg hypochloric acid).
- * Chloride also plays an important role in maintaining cellular equilibrium in respiration by exchanging with bicarbonate across cell membranes (eg *chloride shift* and *reverse chloride shift*).

Chloride

- * A few studies have implicated serum chloride levels as a predictive indicator of mortality from cardiovascular disease and other causes. One study demonstrated an increased risk ratio for cardiovascular death with serum chloride levels below 100 mmol/L.

Fig. 3



Associations between serum chloride levels at baseline and risk of total mortality (a), cardiovascular disease (CVD) mortality (b) and coronary heart disease (CHD) mortality (c) after 10 years of follow-up (BIRNH study).

Is Low Serum Chloride Level a Risk Factor for Cardiovascular Mortality?

Dirk De Bacquer, Guy De Backer, Marc De Buyzere and Marcel Kornitzer
Journal of Cardiovascular Risk 1998 5: 177
 DOI: 10.1177/174182679800500307

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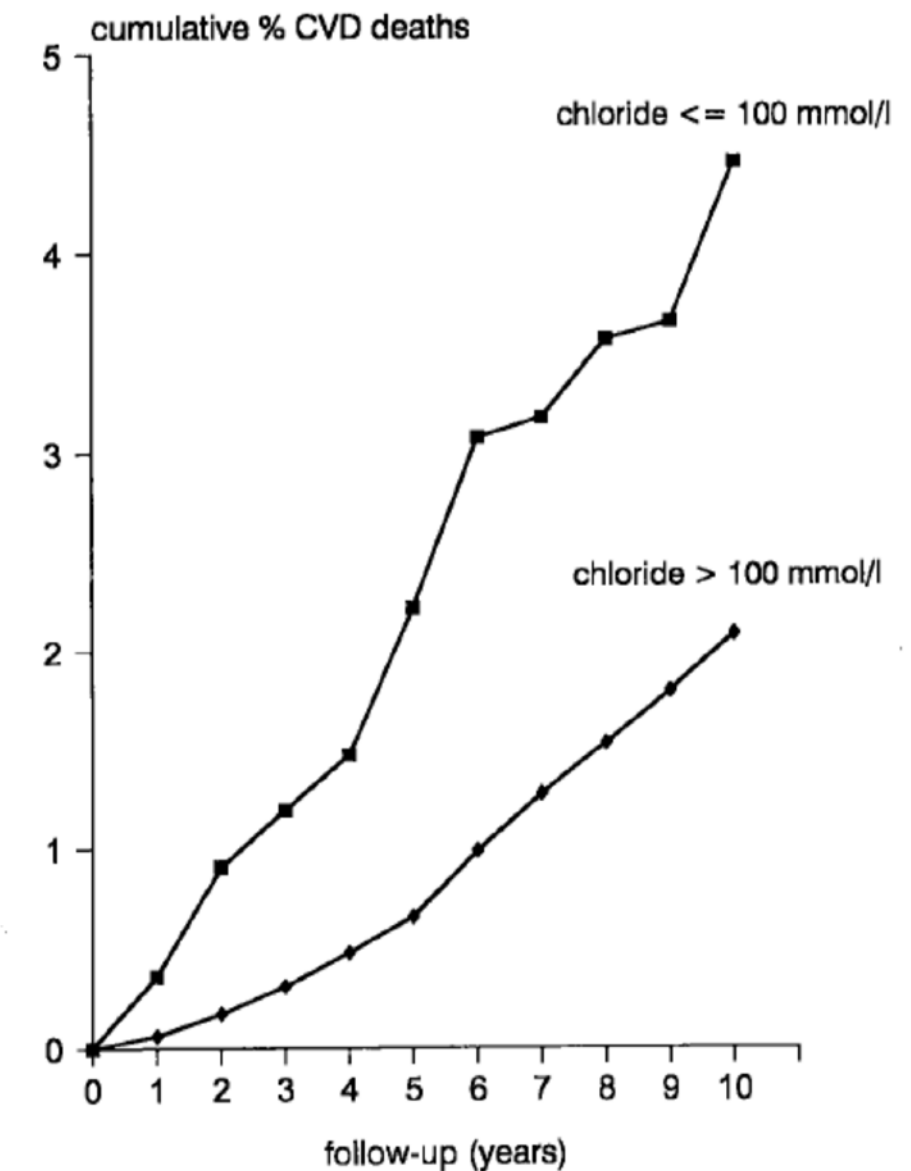
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Fig. 4



Cumulative cardiovascular disease (CVD) mortality rates by initial serum chloride levels in men and women free of CHD (BIRNH study).

Chloride

Traditional Reference range:

- * 96-109 mg/dL or 96-109 mmol/L

Optimal Reference Range:

- * 102-105 mg/dL or 102-105 mmol/L

Chloride - Elevated

- * **Elevated** – Functional hyperchloremia is often associated with acidosis and, in conjunction with a normal *anion gap* is called hyperchloremic acidosis (rare).

Chloride - Elevated

Cause	Reason	Additional Inquiry
Kidney dysfunction	Inability to secrete hydrogen ions or reabsorb bicarbonate. Either way, the net result is excess acidity.	Evaluate kidney markers.
Diarrhea	Excessive loss of bicarbonate from plasma tends to draw chloride out of cells to replace the loss of negatively charged ions in the plasma and thus elevated chloride.	Evaluate CO ₂ .
Dehydration	Similar to other electrolytes, if plasma volume has decreased, hemoconcentration may make it appear chloride is elevated.	Evaluate other dehydration markers.
Hyperparathyroidism	Associated with kidney dysfunction and increased renal tubular acidosis, a cause of high chloride.	Evaluate calcium. If low, consider running PTH.
Hyperventilation	Rapid breathing can cause a shift in pH and thus chloride levels. However this tendency is often compensated and may have minimal effects on serum chloride levels.	Evaluate respiration rate.

Chloride - Decreased

- * **Decreased** – Functional *hypochloremia* is often associated with an increase of bicarbonate levels and thus an overly alkaline state.

Chloride - Decreased

Cause	Reason	Additional Inquiry
Severe vomiting	Loss of hydrochloric acid (hydrogen and chloride) can lead to low chloride levels.	Client history.
Chronic respiratory acidosis (hypoventilation)	Bicarbonate will be elevated in an attempt to compensate for low pH. Chloride will be lost by the kidneys while increasing bicarbonate levels.	Evaluate respiration rate.
Metabolic alkalosis	Bicarbonate and chloride are the only two anions in blood and thus when one goes up (eg bicarbonate) the other will go down (eg chloride). Thus in metabolic alkalosis, low chloride levels are observed.	
Adrenal insufficiency	Aldosterone reabsorbs sodium and chloride ions. Thus if aldosterone is low, chloride will like be decreased.	Evaluate adrenal function.

Sodium-chloride Difference and Metabolic Syndrome: A Population-based Large-scale Cohort Study

Toshihiro Kimura¹, Yoshitaka Hashimoto¹, Muhei Tanaka¹, Mai Asano¹, Masahiro Yamazaki¹,
Yohei Oda¹, Hitoshi Toda², Yoshinori Marunaka³, Naoto Nakamura¹ and Michiaki Fukui¹

Table 3. Odds Ratio of Prevalence of MetS according to Quartiles of Serum Na⁺, Serum Cl⁻ Or Na⁺-Cl⁻ Level.

Serum Na ⁺ (mmol/L)	≤ 140	141-142		143		≥144	
Case/N	91/736	190/1,448		88/734		152/957	
	OR	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Unadjusted OR	1 (Reference)	1.39 (0.91-2.14)	0.1237	1.26 (0.79-2.04)	0.3387	1.82 (1.18-2.84)	0.0068
Adjusted OR*	1 (Reference)	1.07 (0.82-1.40)	0.6163	0.97 (0.71-1.32)	0.8260	1.34 (1.01-1.78)	0.0395
Serum Cl ⁻ (mmol/L)	≤ 99	100-101		102-103		≥104	
Case/N	120/630	164/1,184		150/1,238		87/823	
	OR	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Unadjusted OR	1 (Reference)	0.68 (0.53-0.89)	0.0042	0.59 (0.45-0.76)	< 0.0001	0.50 (0.37-0.68)	< 0.0001
Adjusted OR*	1 (Reference)	0.83 (0.56-1.23)	0.3424	0.86 (0.59-1.29)	0.5005	0.90 (0.58-1.38)	0.6172
Na ⁺ -Cl ⁻ (mmol/L)	≤ 38	39-40		41-42		≥ 43	
Case/N	62/772	151/1,175		156/1,143		152/785	
	OR	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Unadjusted OR	1 (Reference)	1.69 (1.25-2.32)	0.0007	1.81 (1.34-2.49)	0.0001	2.76 (2.02-3.80)	< 0.0001
Adjusted OR*	1 (Reference)	1.34 (0.88-2.06)	0.1740	1.16 (0.76-1.78)	0.5064	1.81 (1.17-2.84)	0.0078

MetS: Metabolic syndrome, Na⁺-Cl⁻: Sodium-chloride difference, OR: Odds ratio, CI: Confidence interval. *: Adjusted for age, sex, body mass index, systolic blood pressure, fasting plasma glucose, triglycerides, high-density lipoprotein cholesterol, creatinine, uric acid and lifestyle factors such as smoking status, habit of alcohol and habit of exercise.

Anion Gap

- * Acidosis is when the pH of the blood drops below 7.35 and alkalosis is when pH increases above 7.45.
- * pH is often calculated using the Henderson-Hasselbach equation, which requires the *partial pressure* of carbon dioxide (gas).
- * Once pH is determined and either acidosis or alkalosis is identified, determining why there is an acid-base disturbance is necessary, and the anion gap can help accomplish this.

Anion Gap

- * The anion gap is calculated using the following equation:
$$(\text{Na}^+) - ([\text{Cl}^-] + [\text{HCO}_3^-])$$
- * The anion gap can either be elevated, normal, or low, all with acidosis present. Again, the purpose of the anion gap is to help determine the cause of acidosis.

Optimal range

- * 7-12 mmol/L

Anion Gap – Elevated with acidosis

- * **Elevated anion gap** with acidosis is often caused by elevated acids such as ketones or lactic acid and is characterized by the loss of bicarbonate to help buffer the unmeasured anions (eg ketones, lactic acid). It can also be caused by kidney dysfunction (Eg renal tubular acidosis) where acids are not being excreted and bicarbonate is not being reabsorbed. Nutritional reasons for excess lactic acid production include thiamin deficiency, biotin deficiency, or anemic tendencies. An increase in the anion gap can also result from either a decrease in unmeasured cations (eg, hypokalemia, hypocalcemia, hypomagnesemia) or an increase in unmeasured anions (eg, hyperphosphatemia, high albumin levels).

Anion Gap – Normal with acidosis

- * **Normal anion gap** with acidosis is sometimes referred to as hyperchloremic acidosis and is characterized by a relative loss of bicarbonate from the body and a subsequent increase in chloride anions to replace it. Possible causes of this pattern include gastrointestinal loss (Eg diarrhea), kidney dysfunction, hypoadrenal function (eg low aldosterone), and possibly zinc deficiency.

Anion Gap - Decreased

- * **Low anion gap** is most often caused by low albumin levels, as albumin is a negatively charged protein. If albumin levels are below 4.0 it is suggested to use the adjusted anion gap calculation:

adjusted anion gap =

observed anion gap + $0.25 \times ([4.5] - [\text{observed albumin}])$

- * A decrease in the anion gap can also result from either a increase in unmeasured cations (eg, hyperkalemia, hypercalcemia, hypermagnesemia) or an decrease in unmeasured anions (eg, hypophosphatemia, low albumin levels).

Serum anion gap, bicarbonate and biomarkers of inflammation in healthy individuals in a national survey

Wildon R. Farwell MD MPH, Eric N. Taylor MD MSc

Table 2: Adjusted differences in inflammatory biomarkers, by quartile of serum anion gap

Biomarker	Quartile of serum anion gap; difference in biomarker (95% CI)				p value for trend
	Quartile 1 (< 10 mmol/L)	Quartile 2 (10–11 mmol/L)	Quartile 3 (12–13 mmol/L)	Quartile 4 (> 13 mmol/L)	
Leukocyte count, $\times 10^9$ /L					
Adjusted for age	Referent	0.5 (0.3 to 0.8)	0.9 (0.6 to 1.1)	1.0 (0.7 to 1.2)	< 0.01
Adjusted for multiple variables*	Referent	0.5 (0.3 to 0.7)	0.9 (0.6 to 1.1)	1.0 (0.7 to 1.3)	< 0.01
C-reactive protein level, nmol/L					
Adjusted for age	Referent	0.2 (–3.6 to 4.0)	3.0 (–1.5 to 7.5)	4.4 (0.6 to 8.1)	0.01
Adjusted for multiple variables*	Referent	1.4 (–2.3 to 5.2)	7.5 (3.2 to 11.8)	10.9 (6.2 to 15.6)	< 0.01

Note: CI = confidence interval.

*Adjusted for age, sex, body mass index, ethnic background, use of alcohol, serum levels of cotinine, albumin, creatinine, uric acid and urea, and dietary protein intake.

Hated with increased mortality and the development of chronic disease. A recent study reported that all-cause mortality increased 11% for every increase of 1.0 × 10⁹/L in leukocyte count above 3.5 × 10⁹/L.¹ Other studies also reported a significant relation between mortality and

tus of the general population of the United States. Participants
408

From the Massachusetts Veterans Epidemiology Research and Information Center, VA Boston Healthcare System, and the Division of Aging, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School (Farwell);

Original Article: Metabolism

Serum bicarbonate, anion gap and insulin resistance in the National Health and Nutrition Examination Survey

W. R. Farwell*† and E. N. Taylor‡

*Massachusetts Veterans Epidemiology Research and Information Center, VA Boston Healthcare System, †Division of Aging and ‡Renal Division and Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Accepted 10 March 2008

Abstract

Aims Metabolic acidosis may contribute to the development of insulin resistance. To date, there have been no population-

“Lower bicarbonate and higher anion gap are independently associated with insulin resistance.”

of bicarbonate had fasting insulin 12.76 pmol/l lower [95% confidence interval (CI) 5.96, 19.55; P for trend < 0.01] than those in the lowest quartile. Participants in the highest quartile of anion gap had fasting insulin 4.39 pmol/l higher (95% CI 0.47, 8.31; P for trend < 0.01) than those in the lowest quartile.

Conclusions Lower bicarbonate and higher anion gap are independently associated with insulin resistance. Further research is needed to elucidate the relations between organic acid production, insulin resistance, and the pathogenesis of Type 2 diabetes.

Diabet. Med. 25, 798–804 (2008)

Keywords anion gap, epidemiology, insulin resistance, metabolic acidosis, NHANES

Abbreviations BMI, body mass index; CI, confidence interval; GFR, glomerular filtration rate; HOMA-IR, homeostasis model assessments for insulin resistance; MDRD, Modification of Diet in Renal Disease Study; MFFM, glucose disposal rates (M) corrected for fat-free mass (FFM); NHANES, National Health and Nutrition Examination Survey

Introduction

Insulin resistance plays an important role in Type 2 diabetes and is an independent risk factor for cardiovascular disease [1–4]. The pathogenesis of insulin resistance is poorly understood and remains under active investigation. It has been suggested that metabolic acidosis may contribute to the development of

Increased renal net acid excretion and higher levels of organic acid production have been reported in individuals with Type 2 diabetes. A recent cross-sectional study comparing the 24-h urine composition of individuals with Type 2 diabetes (and high levels of fasting insulin) with that of normal volunteers has reported lower urinary pH in those with diabetes, even after adjustment for age, body size, creatinine clearance and

Osmolarity

- * Osmolarity refers to the concentration of given solutes (Eg electrolytes, proteins, glucose, urea) dissolved in an aqueous solution, such as the blood or plasma.
- * Serum osmolarity can therefore help determine the relative concentration or dilution of the blood.
- * The osmolarity of blood can be measured directly, or can be calculated using other markers found on a blood chemistry.

Osmolarity

- * Of the many different calculations for osmolarity, these three have provided the most accurate results when compared to actual osmolarity of the blood:
 - * $2 \times \text{Na} + .9 \times \text{glucose} + .93 \times .5 \times \text{urea} + 8$
 - * $1.897 \times \text{Na} + \text{glucose} + \text{urea} \times .5 + 13.5$
 - * $1.9 \times (\text{Na} + \text{K}) + \text{glucose} + \text{urea} \times .5 + 5$
- * While any of these calculation will likely prove helpful in estimating one's serum osmolarity, one could also consider using all three calculations and determining the mean osmolarity of the three.

Osmolarity

- * Elevated osmolarity indicates relatively concentrated blood and can therefore be an indication of dehydration.
- * This is important because blood is considered to be an extra cellular fluid and, because the body tends to balance the osmolarity of fluid inside and outside of cells, can be an indication of cellular hydration as well.

Osmolarity

- * For example if serum osmolarity is elevated (eg concentrated), water will tend to be pulled out of cells in an attempt to dilute it causing cellular dehydration and thus cellular dysfunction.
- * On the other hand, low serum osmolarity will tend to drive water into cells in an attempt to concentrate the blood, leading to cellular swelling, which also causes cellular dysfunction

Osmolarity

Traditional Reference range:

- * 285-295 mOsm/kg

Optimal Reference Range:

- * 288-292 mOsm/kg

Osmolarity - Elevated

- * **Elevated** – Elevated serum osmolarity indicates overly concentrated blood, of which a primary cause is low plasma water, or dehydration.

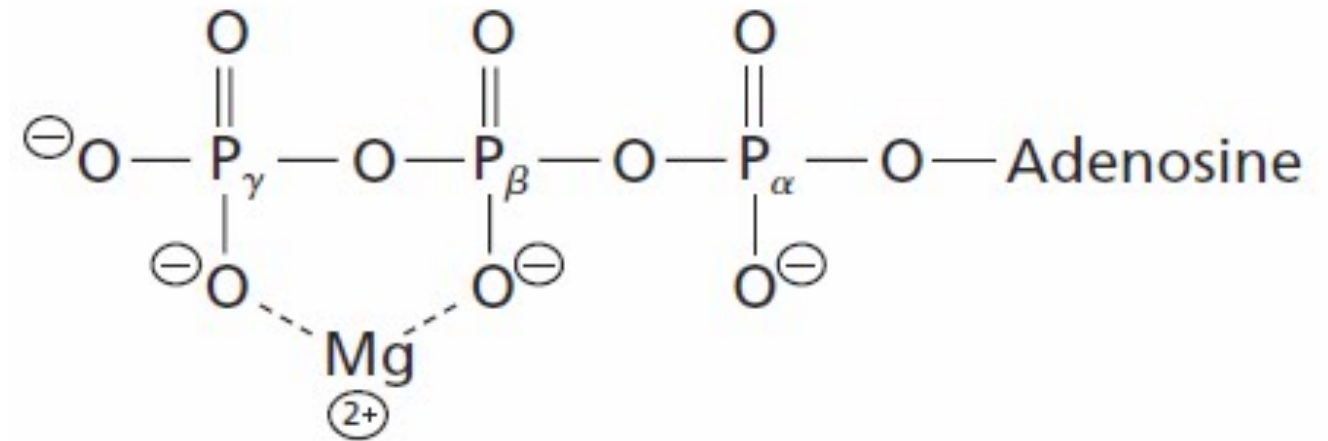
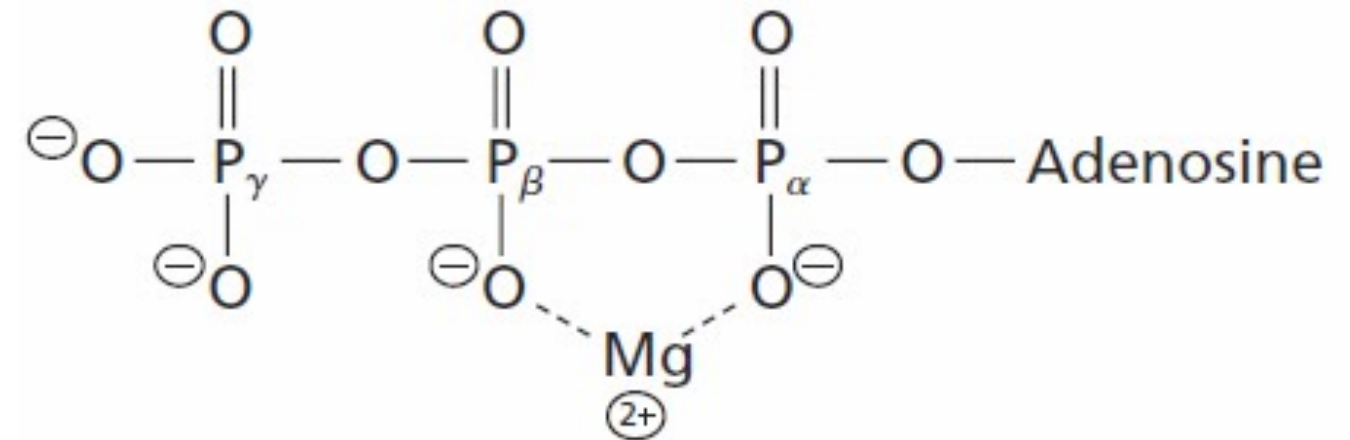
Cause	Reason	Additional Inquiry
Dehydration	Poor water intake	Client history.
Elevated urea	High protein intake.	Client history.
Elevated glucose	Poorly managed blood sugar.	Evaluate blood sugar markers.
Kidney dysfunction	Inability to filter and excrete urea causes urea to remain elevated in plasma and increase osmolarity.	Evaluate kidney markers.

Osmolarity - Decreased

Cause	Reason	
Overhydration	Excess, rapid water consumption can cause this, though likely rare.	Client history.
Excess ADH production	Anti-diuretic hormone, produced from the posterior pituitary, signals the kidneys to retain water without reabsorbing electrolytes, with the net effect being lowering serum osmolarity.	
Decreased sodium	Most often caused by diuretics or other medications, increasing sodium excretion by the kidneys.	

Magnesium

- * Magnesium is involved with the activation of over 300 enzymes in the body, thus giving it profound biochemical importance.
- * One particular role of magnesium is complexing with ATP, allowing ATP to be used for energy production. It is the fourth most abundant cation in the body, but intracellularly is second only to potassium.



Magnesium

- * Regulation of magnesium includes a number hormones.
- * Parathyroid hormone increases magnesium reabsorption in the kidneys as well as increases magnesium absorption in the intestines.
- * Vitamin D on the other hand decreases magnesium absorption in the intestines.

Magnesium

- * Magnesium has interrelationship with many other nutrients.
- * For example both high magnesium and calcium inhibit parathyroid hormone release, but magnesium is also required for parathyroid hormone release.
- * Magnesium is also needed for proper use of parathyroid hormone on target cells. Magnesium has also been called 'nature's calcium channel blocker' and can influence calcium's transport across cell membranes.
- * Magnesium is required for hydroxylation of vitamin D in the liver.
- * Magnesium can inhibit phosphorus absorption in the intestine, and also has a relationship with potassium efflux across cell walls, causing hypokalemia.

Magnesium

- * Recent studies have shown an inverse relationship between magnesium, atherosclerosis, and cardiovascular mortality, showing the lower ones serum magnesium levels, the greater the risk of cardiovascular mortality.
- * Low magnesium levels have also been shown to decrease pancreatic exocrine function, which may cause decrease in digestive enzyme production and secretion.
- * Magnesium deficiency is associated with the increased production of ROS and the induction of immune and inflammatory reactions.

Magnesium

- * Serum magnesium levels are decreased in low protein diets, elevated estrogen levels, diabetes, and selenium, vitamin D and vitamin B6 deficiencies.
- * It must be noted that because magnesium is largely an intracellular cation, low serum magnesium levels are suggested to indicate a fairly significant magnesium deficiency and, just because serum magnesium levels are normal, does not mean intracellular levels are sufficient.

Perspective: The Case for an Evidence-Based Reference Interval for Serum Magnesium: The Time Has Come¹⁻⁵

Rebecca B Costello,^{6*} Ronald J Elin,⁷ Andrea Rosanoff,⁶ Taylor C Wallace,⁸ Fernando Guerrero-Romero,⁹ Adela Hruby,¹⁰ Pamela L Lutsey,¹¹ Forrest H Nielsen,¹² Martha Rodriguez-Moran,⁹ Yiqing Song,¹³ and Linda V Van Horn¹⁴

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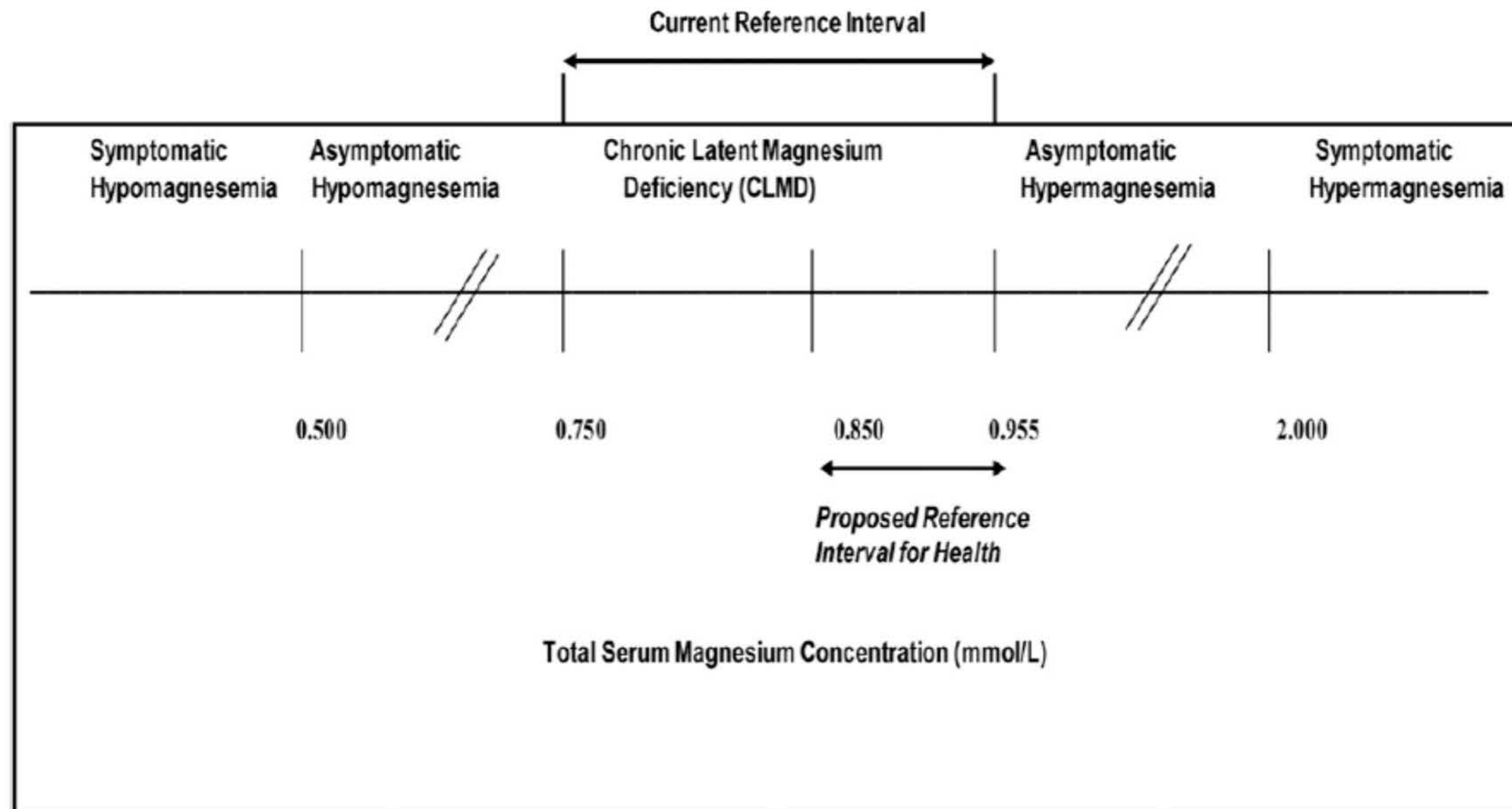


FIGURE 6 Current and proposed clinical cut-offs of the serum total magnesium concentration for assessing magnesium status.

Serum nutritional markers for prediction of pancreatic exocrine insufficiency in chronic pancreatitis

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ABSTRACT

Background: Methods for evaluation of pancreatic exocrine insufficiency (PEI) are expensive, labor intensive, and not available at many institutions. The aim of this study was to investigate if PEI in chronic pancreatitis (CP) can be predicted by nutritional markers in blood.

Methods: A retrospective analysis of a prospectively collected database of CP patients was performed. Diagnosis of CP was based on endoscopic ultrasonography or magnetic resonance imaging. PEI was

Magnesium below 2.05 mg/dL detected PEI with a sensitivity, specificity and positive and negative predictive values of 0.88 (95% confidence interval, 0.66e0.97), 0.66 (0.48e0.80), 0.58 (0.39e0.75) and 0.91 (0.73e0.98), respectively.

Conclusion: Serum nutritional markers can be used to predict the probability of PEI in CP and provide guidance in decisions on enzyme replacement therapy.

eventually develop PEI after a median time of 12 years after the diagnosis of chronic pancreatitis [1]. It is generally accepted that

fact that PEI can be treated with oral administration of pancreatic enzyme preparations. Gold standard for demonstration of PEI is 3 days fecal fat quantification with calculation of the coefficient of fat absorption [3]. The method requires the patient to keep a strict diet of 100 g fat per day during five days and to collect and store the complete volume of feces during the last three days. Dietary restrictions and the handling of large volumes of feces make the method cumbersome and unpleasant for both patients and laboratory personnel. As a consequence, the use of fecal fat quantification is nowadays limited to a few specialized centers. Other

Abbreviations: PEI, pancreatic exocrine insufficiency; CP, chronic pancreatitis; ¹³C-MTG, ¹³C-mixed triglycerides; s-MRCP, secretin enhanced magnetic resonance cholangiopancreatography; MRI, magnetic resonance imaging; CI, confidence interval; SD, standard deviation.

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Magnesium – Serum (Not RBC)

Traditional Reference range:

- * 1.6 – 2.6 mg/dL or .65 - 1.07 mmol/L

Optimal Reference Range:

- * 2.0 – 2.3 mg/dL or .86-.95 mmol/L

Magnesium - Elevated

- * **Elevated** – Hypermagnesemia is relatively rare and is most common with kidney dysfunction

Cause	Reason	Additional Inquiry
Hemolysis	Because magnesium is an intracellular ion, if red blood cells rupture prematurely, magnesium will increase in the blood.	Evaluate potassium and CBC markers.
Kidney dysfunction	Poor filtration by the kidneys will increase plasma magnesium levels.	Evaluate kidney markers.
Hyperparathyroidism	PTH increases magnesium absorption in the intestines leading to elevated magnesium levels.	Evaluate calcium.
Adrenal insufficiency	Poor magnesium clearance from the kidneys.	Evaluate sodium and potassium.
Dehydration	Hemoconcentration.	Evaluate other dehydration markers.
Hypothyroidism	Unknown mechanism	Evaluate thyroid markers.

Magnesium - Decreased

Cause	Reason	
Poor intake		Diet journal
Poor absorption	Magnesium absorption can be inhibited by phytates, oxalates, calcium, phosphorus, or free fatty acids in the intestines.	Diet journal.
Alcoholism	Alcohol stimulates magnesium excretion from the kidneys.	Client history.
Diarrhea	Excessive loss, including loss of other fluids.	Client history.
Diabetes	Excessive loss via the kidneys.	
Hypercalcemia	Excess magnesium loss from kidneys.	Evaluate calcium levels.
Hyperadrenal function	Two mechanisms. One, magnesium inhibits aldosterone production. Thus if magnesium is low, aldosterone may be elevated. Two, aldosterone causes magnesium loss.	Evaluate adrenal markers.

Calcium

- * Calcium, most well-known for its role in bone and teeth, has a number of other important physiological roles in the body including clotting, neurotransmitter release, muscle contraction, enzyme activity and membrane permeability.
- * Bones and teeth contain up to 99% of the body's totally calcium, which constitutes roughly 1-2% of the total weight of the body.

Calcium

- * Because of these many important roles, calcium is the most tightly regulated ion in blood.
- * In blood, approximately half of the calcium is in it ionized (free) form, while the other half is found bound to proteins, such as albumin.
- * On a blood chemistry, unless it states otherwise (Eg ionized or free), the calcium listed is bound calcium.

Calcium

- * Because serum calcium on a blood chemistry is bound, if albumin levels are decreased, so too will serum calcium levels. Thus, the following formula is used to correct total serum calcium levels whenever albumin is low:

$$\text{Corrected calcium} = ([4.5 - \text{observed albumin}) \times 0.8 \text{ mg/dL}) + \text{observed calcium}$$

Calcium

- * Another role of calcium is in the calcification of tissues, such as in atherosclerosis, and generally seems to occur after tissue damage or injury.
- * This not necessarily due to dietary calcium but rather the availability and distribution of calcium in the body, and its affinity to bind to proteins.
- * There are a number of important interactions with hormone and other nutrients that dictate how calcium is used in the body, including parathyroid hormone, calcitonin, vitamin D, vitamin K, magnesium, phosphorus, and more.
- * It is because of these relationships with other nutrients, and what seems to be widespread deficiency in some of these nutrients, that excess tissue calcification may occur.

Calcium Regulation

- * Parathyroid hormone, released by the parathyroid glands, is released when calcium levels become low.
- * PTH stimulates:
 - * calcium reabsorption from bone,
 - * increased calcium absorption from the intestines, and
 - * decreased calcium excretion from the kidneys.
- * PTH also increases phosphorus reabsorption from bone, but simultaneously increases phosphorus excretion from the kidneys.
- * PTH also increases production of active vitamin D from the kidneys, which increases calcium absorption from the intestines.

Free Fatty Acids Alter Calcium Binding: A Cause for Misinterpretation of Serum Calcium Values and Hypocalcemia in Critical Illness*

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ABSTRACT. FFAs are bound with calcium on the albumin molecule. We hypothesized that changes in circulating FFA levels during critical illness altered calcium-albumin binding. We found that serum from both normal subjects and critically ill patients contained an ether-extractable factor which lowered ionized calcium concentrations and increased albumin-calcium binding. This factor was found in higher concentrations in serum from ill patients. Oleic acid and palmitic acid increased albumin-calcium binding from 2–28% in a dose-dependent manner when added *in vitro* to calcium-albumin solutions. Scatchard analysis demonstrated that 0.1 mM oleic acid increased the number of calcium-binding sites on the albumin molecule (from three to five sites per molecule) without altering binding affinity. A similar effect was found when we performed Scatchard analyses

of ether extracts in serum from three critically ill patients (number of calcium-binding sites increased from three to six). We also found that lipid infusions (during parenteral nutrition) lowered mean serum ionized calcium values in six critically ill patients [4.6 ± 0.2 ($\pm$ SEM) to 4.1 ± 0.2 mg/dL; $P < 0.05$]. These data support the concept that FFAs increase calcium binding to the albumin molecule. Alterations in FFA concentrations during critical illness may contribute to the poor correlation between corrected total serum calcium and ionized calcium concentrations in critically ill patients. In addition, acute elevations in circulating FFA concentrations may contribute to hypocalcemia in patients with defects in bone calcium mobilization. (*J Clin Endocrinol Metab* 64: 1010, 1987)

ABNORMAL total serum calcium concentrations are found in more than two thirds of critically ill patients (1–13) (Zaloga, G. P., and B. Chernow, submitted). When the serum ionized calcium concentration is measured, about 12% of these patients are hypocalcemic

FFAs are carried in the circulation bound to albumin. Levels of these acids increase during illness (17, 18) and after iv lipid infusions. Thus, the experiments performed in this study were designed to evaluate the effect of FFA alterations on calcium binding in serum from patients with critical illness.

Serum calcium level is associated with metabolic syndrome in the general population: FIN-D2D study

Table 1 Basic characteristics of study population according to the serum total calcium (mmol/l). Data are mean (s.d.).

	Serum calcium (mmol/l)				<i>P</i> value*
	≤2.39	2.40–2.49	2.50–2.59	≥2.60	
Men					
<i>n</i>	154	606	487	97	
Age	62 (8)	60 (9)	60 (8)	61 (8)	0.28
BMI (kg/m ²)	27.2 (3.9)	27.4 (4.5)	27.5 (4.0)	28.1 (3.7)	0.015
Waist (cm)	99 (12)	100 (12)	100 (11)	103 (11)	0.044
Systolic blood pressure (mmHg)	137 (19)	136 (18)	141 (19)	141 (19)	0.001
Diastolic blood pressure (mmHg)	82 (11)	82 (10)	84 (10)	85 (9)	0.003
Fasting plasma glucose (mmol/l)	6.2 (0.9)	6.4 (1.2)	6.5 (1.3)	6.7 (1.8)	<0.001
2 h plasma glucose (mmol/l)	6.8 (2.5)	7.0 (3.0)	7.4 (2.9)	8.2 (4.8)	<0.001
Total cholesterol (mmol/l)	5.0 (1.0)	5.3 (1.0)	5.5 (1.0)	5.4 (1.1)	<0.001
HDL-cholesterol (mmol/l)	1.27 (0.30)	1.31 (0.31)	1.34 (0.33)	1.30 (0.32)	0.11
Triglycerides (mmol/l)	1.35 (0.67)	1.40 (0.85)	1.58 (1.17)	1.80 (1.16)	<0.001
S-GFR (ml/min)	95 (28)	94 (25)	94 (24)	94 (21)	0.98
Dietary calcium (mg/day)	1463 (628)	1508 (685)	1520 (681)	1760 (939)	<0.007
Dietary vitamin D (μg/day)	11.0 (7.0)	11.1 (6.2)	11.5 (6.8)	13.5 (17.2)	0.023
Women					
<i>n</i>	183	625	536	138	
Age	54 (9)	58 (8)	61 (8)	62 (7)	<0.001
BMI (kg/m ²)	26.5 (6.0)	27.4 (5.1)	27.8 (5.2)	28.9 (5.6)	<0.001
Waist (cm)	88 (14)	90 (13)	91 (13)	94 (14)	<0.001
Systolic blood pressure (mmHg)	128 (19)	135 (19)	137 (19)	141 (17)	<0.001
Diastolic blood pressure (mmHg)	78 (9)	81 (9)	80 (8)	81 (9)	0.004
Fasting plasma glucose (mmol/l)	5.9 (1.3)	6.0 (1.0)	6.1 (1.0)	6.2 (1.0)	<0.001
2 h plasma glucose (mmol/l)	6.5 (2.3)	6.8 (2.2)	7.3 (2.5)	7.5 (2.6)	<0.001
Total cholesterol (mmol/l)	5.2 (0.8)	5.5 (0.9)	5.7 (1.0)	5.7 (1.1)	<0.001
HDL-cholesterol (mmol/l)	1.52 (0.34)	1.54 (0.33)	1.56 (0.33)	1.58 (0.37)	0.12
Triglycerides (mmol/l)	1.12 (0.60)	1.22 (0.57)	1.36 (0.63)	1.44 (0.65)	<0.001
S-GFR (ml/min)	88 (27)	83 (23)	80 (23)	81 (22)	0.003
Dietary calcium (mg/day)	1518 (683)	1456 (587)	1475 (632)	1563 (660)	0.55
Dietary vitamin D (μg/day)	8.2 (4.0)	8.3 (4.3)	9.0 (5.2)	9.0 (3.9)	0.01

Serum calcium is independently associated with insulin sensitivity measured with euglycaemic–hyperinsulinaemic clamp in a community-based cohort

Table 1 Baseline characteristics of the total cohort and for quartiles of serum calcium concentration

Variable	Total cohort	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Age (years)	71.0±0.58	71.1±0.58	71.0±0.59	70.9±0.54	70.9±0.60
Serum calcium (mmol/l) ^a	2.33±0.09	2.22±0.09	2.31±0.05	2.36±0.05	2.44±0.06
Serum calcium range (mmol/l) ^a	1.62–2.85	1.62–2.28	2.29–2.33	2.34–2.39	2.40–2.85
Serum albumin (g/l)	43.1±2.6	43.7±2.5	43.6±2.6	42.8±2.4	42.4±2.7
BMI (kg/m ²)	26.2±3.4	26.5±3.2	26.4±3.5	25.9±3.2	26.0±3.5
Fasting plasma glucose (mmol/l)	5.75±1.4	5.71±1.4	5.76±1.5	5.84±1.5	5.68±1.2
Fasting serum insulin (pmol/l)	88.2±49	88.2±51	86.1±48	83.3±47	90.3±50
M/I					
mg kg ⁻¹ min ⁻¹ (mU/l) ⁻¹ ×100	5.06±2.5	5.11±2.4	5.14±2.6	5.20±2.5	4.79±2.3
mg kg ⁻¹ min ⁻¹ (pmol/l) ⁻¹ ×100	0.73±0.36	0.74±0.35	0.74±0.37	0.75±0.36	0.69±0.33
M (mg min ⁻¹ kg ⁻¹)	5.20±2.1	5.22±2.0	5.21±2.1	5.31±2.1	5.04±2.1
HOMA insulin sensitivity	3.34±2.4	3.36±2.6	3.27±2.3	3.18±2.3	3.54±2.3
EIR	13.7±11	13.2±11	13.3±11	13.6±11	14.7±11
Serum creatinine (μmol/l)	93.7±14	92.4±14	92.2±12	94.1±13	96.1±15
Serum phosphate (mmol/l)	0.96±0.21	0.94±0.2	0.95±0.2	0.97±0.2	0.99±0.3
Coffee (cups per day)	3.36±1.6	3.3±1.6	3.3±1.5	3.3±1.7	3.5±1.7
Tea (cups per day)	0.67±0.7	0.68±0.7	0.70±0.7	0.70±0.6	0.60±0.6
Dietary calcium (mg/day)	965±337	975±351	981±325	961±326	943±347
Alcohol (g/day)	1.08±0.62	1.11±0.61	1.06±0.62	1.11±0.63	1.05±0.61
Physical activity level: <i>n</i> (%)					
Sedentary	31 (3.23)	5 (2.07)	7 (3.0)	8 (3.17)	10 (4.48)
Moderate	326 (33.9)	87 (36.1)	78 (33.5)	86 (34.1)	72 (32.3)
Regular	546 (57.1)	139 (57.7)	135 (57.9)	139 (55.2)	130 (58.3)
Athletic	55 (5.72)	10 (4.15)	13 (5.6)	19 (7.54)	11 (4.9)
Smoking: <i>n</i> (%)	196 (20.4)	48 (19.9)	43 (18.5)	44 (17.5)	60 (26.9)

Data are mean±SD

n=961

^aAlbumin-corrected serum calcium

Serum Calcium Levels Are Associated with Novel Cardiometabolic Risk Factors in the Population-Based CoLaus Study

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Abstract

Background: Associations of serum calcium levels with the metabolic syndrome and other novel cardio-metabolic risk factors not classically included in the metabolic syndrome, such as those involved in oxidative stress, are largely unexplored. We analyzed the association of albumin-corrected serum calcium levels with conventional and non-conventional cardio-metabolic risk factors in a general adult population.

Methodology/Principal Findings: The CoLaus study is a population-based study including Caucasians from Lausanne, Switzerland. The metabolic syndrome was defined using the Adult Treatment Panel III criteria. Non-conventional cardio-metabolic risk factors considered included: fat mass, leptin, LDL particle size, apolipoprotein B, fasting insulin, adiponectin, ultrasensitive CRP, serum uric acid, homocysteine, and gamma-glutamyltransferase. We used adjusted standardized multivariable regression to compare the association of each cardio-metabolic risk factor with albumin-corrected serum calcium. We assessed associations of albumin-corrected serum calcium with the cumulative number of non-conventional cardio-metabolic risk factors. We analyzed 4,231 subjects aged 35 to 75 years. Corrected serum calcium increased with both the number of the metabolic syndrome components and the number of non-conventional cardio-metabolic risk factors, independently of the metabolic syndrome and BMI. Among conventional and non-conventional cardio-metabolic risk factors, the strongest positive associations were found for factors related to oxidative stress (uric acid, homocysteine and gamma-glutamyltransferase). Adiponectin had the strongest negative association with corrected serum calcium.

Conclusions/Significance: Serum calcium was associated with the metabolic syndrome and with non-conventional cardio-metabolic risk factors independently of the metabolic syndrome. Associations with uric acid, homocysteine and gamma-glutamyltransferase were the strongest. These novel findings suggest that serum calcium levels may be associated with cardiovascular risk via oxidative stress.

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Competing Interests: VM is a full-time employee of GSK and the CoLaus study was sponsored in part by GlaxoSmithKline. There are no patents, products in development or marketed products to declare. MB is an Academic Editor for PLoS ONE. This does not alter the authors' adherence to all the PLoS ONE policies on sharing data and materials.

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† These authors contributed equally to this work.

Calcium

Traditional Reference range:

- * 8.5-10.6 mg/dL

Optimal Reference Range:

- * 9.4 – 9.8 mg/dL or 2.35-2.45 mmol/L

Calcium - Elevated

Cause	Reason	Additional Inquiry
Alkalosis	Increases binding of calcium to proteins and thus will increase calcium levels on a blood chemistry. Due to this binding however, patients will experience symptoms of hypocalcemia.	Evaluate CO2 for evidence of alkalosis.
Kidney dysfunction	Poor filtration by the kidneys will increase plasma calcium levels.	Evaluate kidney markers.
Hyperparathyroidism	PTH increases magnesium absorption in the intestines leading to elevated magnesium levels.	Evaluate calcium.
Metastasis (cancer)	Some cancers will increase calcium, though this will likely be very elevated levels.	Correlate with elevated platelets and possibly alkaline phosphatase.
Excess Vitamin D intake	High vitamin D levels increase calcium absorption in the intestines and decrease excretion in the kidneys.	Client history. Evaluate vitamin D levels.
Adrenal insufficiency	Mechanism unclear.	Evaluate adrenal markers.
Excess vitamin A intake	Increase calcium reabsorption from bone.	Client history.

Calcium - Decreased

Cause	Reason	
Poor intake and/or absorption	Fiber, phytates, oxalates, excessive zinc and magnesium, and unabsorbed fatty acids can negatively impact calcium absorption in the intestines.	Diet journal.
Hypoparathyroidism	When calcium levels drop, PTH should increase to raise calcium levels. If PTH is low, calcium will remain low.	Run PTH.
Vitamin D deficiency	Vitamin D increases calcium absorption in the intestines. Thus if vitamin D is low, calcium may be low as well.	Evaluate vitamin D levels.
Magnesium deficiency	Magnesium is required for PTH synthesis and release. Thus if magnesium is low, calcium may be low due to lowered PTH.	Evaluate magnesium levels.
Kidney dysfunction		

Phosphorus

- * Phosphorus is an important mineral for life in that it is a major component of the phospholipid membrane surrounding all cells of the body, is involved in RNA and DNA synthesis and coding, is part of Adenosine Triphosphate (ATP) and activates enzymes.
- * Phosphorus is also the primary intracellular buffer to help maintain acid-base balance.
- * Extracellularly, phosphate is also important for the mineralization of bone.
- * Among the inorganic elements, phosphorus is second only to calcium in abundance in the body.

Lower concentrations of serum phosphorus within the normal range could be associated with less calcification of the coronary artery in Koreans with normal renal function¹⁻³

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TABLE 4
Associations between coronary calcium scores (compared with 0) and mean baseline serum phosphorus concentrations¹

Phosphorus subgroups	Agatston score					
	>0 to ≤10		>10 to ≤100		>100	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Unadjusted						
≤3.3 mg/dL (reference)	1.00		1.00		1.00	
>3.3 to ≤3.6 mg/dL	0.50 (0.24, 1.07)	0.075	0.42 (0.20, 0.86)	0.019	0.79 (0.35, 1.79)	0.571
>3.6 to ≤3.9 mg/dL	0.32 (0.13, 0.79)	0.014	0.53 (0.25, 1.10)	0.089	1.24 (0.56, 2.78)	0.597
>3.9 mg/dL	1.24 (0.56, 2.78)	0.597	0.54 (0.26, 1.13)	0.101	1.59 (0.73, 3.47)	0.246
Adjusted ²						
≤3.3 mg/dL (reference)	1.00		1.00		1.00	
>3.3 to ≤3.6 mg/dL	0.69 (0.31, 1.57)	0.377	0.65 (0.29, 1.47)	0.297	1.94 (0.72, 5.23)	0.189
>3.6 to ≤3.9 mg/dL	0.47 (0.18, 1.25)	0.130	0.93 (0.40, 2.17)	0.874	3.89 (1.43, 10.63)	0.008
>3.9 mg/dL	0.63 (0.26, 1.53)	0.311	0.78 (0.34, 1.82)	0.566	3.17 (1.19, 8.41)	0.021

¹ A baseline-category logit model was used to calculate the ORs and 95% CIs.

² Adjusted for age, sex, BMI, diabetes, hypertension, systolic blood pressure, diastolic blood pressure, family history of cardiovascular disease, corrected serum calcium, calcium-phosphorus product, serum glucose, total:HDL cholesterol, and triglyceride. The overall *P* value for the adjusted model was 0.049.

Relation of Serum Phosphorus Levels to Ankle Brachial
Pressure Index (From the Third National Health and Nutrition
Examination Survey)

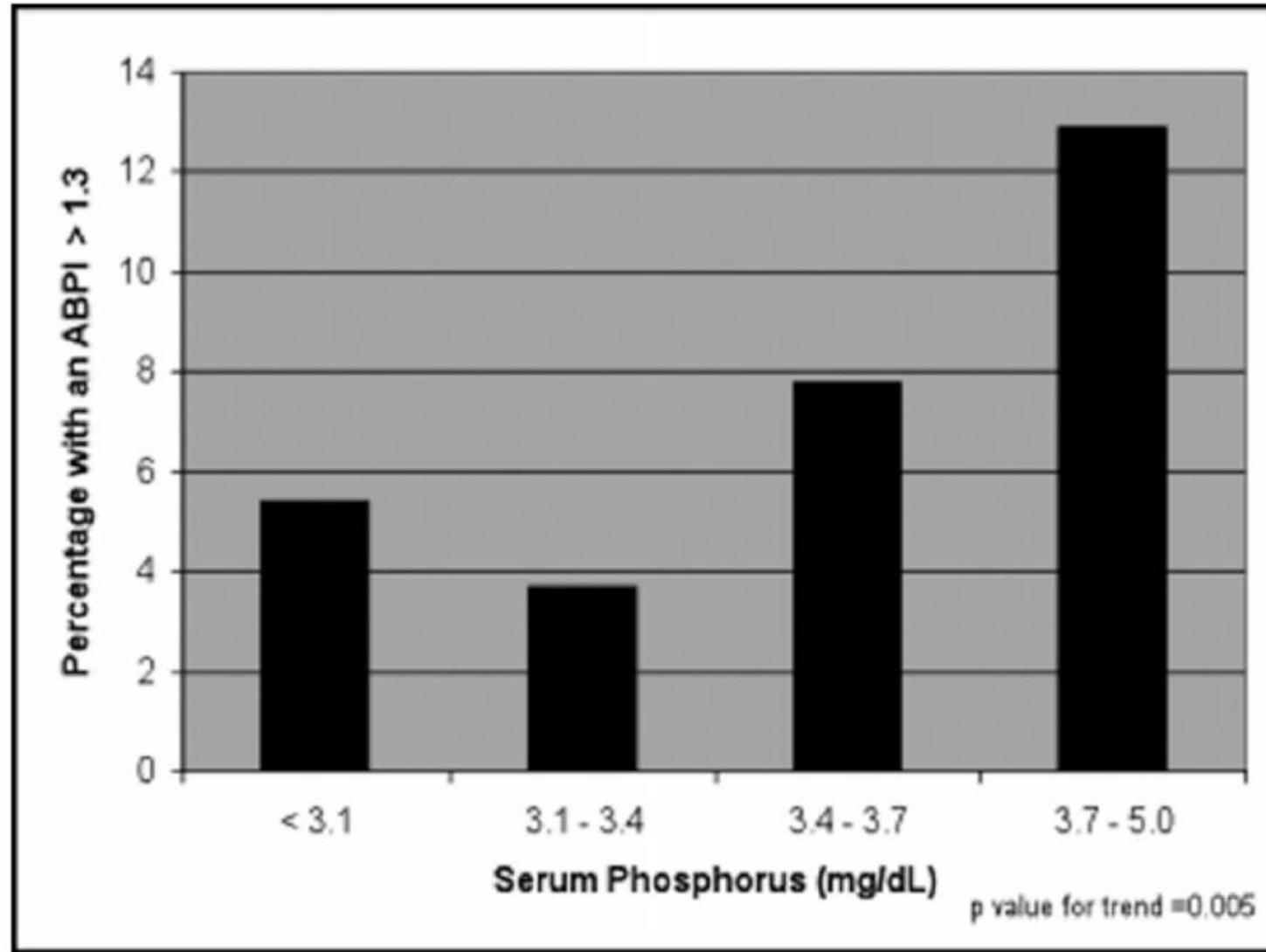


Figure 1.
Prevalence of High Ankle Brachial Pressure Index (defined as ABPI >1.3) According to
Quartiles of Serum Phosphorus Levels

Serum phosphorus and association with anemia among a large diverse population with and without chronic kidney disease

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Among 155 974 individuals, 4.1% had moderate anemia and 12.9% had mild anemia. Serum phosphorus levels ≥ 3.5 mg/dL were associated with both mild and moderate anemia. Moderate anemia OR (95% confidence interval) was 1.16 (1.04–1.29) for every 0.5 mg/dL phosphorus increase and 1.26 (1.07–1.48) in the highest versus middle phosphorus tertile.

The population-based phosphorus tertiles ranged as follows: 1.5–3.1 mg/dL (Q1), 3.2–3.6 mg/dL (Q2) and 3.7–6.2 mg/dL

≥ 3.5 mg/dL were associated with both mild and moderate anemia. Moderate anemia OR (95% confidence interval) was 1.16 (1.04–1.29) for every 0.5 mg/dL phosphorus increase and 1.26 (1.07–1.48) in the highest versus middle phosphorus tertile. Additional independent anemia risk factors, including female sex, Asian race, diabetes, low albumin and low iron saturation, were observed, but did not alter the anemia–phosphorus association.

Conclusions. Higher phosphorus levels were associated with a greater likelihood for anemia in a population with early CKD and normal kidney function. Phosphorus may be a biomarker for anemia and may affect aspects of hematopoiesis.

Keywords: anemia risk, epidemiology, serum phosphorus

anemia independent of other mineral bone disease components [6, 7]. Along with anemia, hyperphosphatemia has been linked with lower bone mineral density in the peritoneal dialysis population [5]. It is also associated with high levels of PTH, which has been shown to inhibit erythropoiesis, induce hemolysis and cause bone marrow fibrosis in CKD states [8–12]. In addition, FGF-23 and its interplay with klotho, vitamin D and iron have been implicated with anemia [13–15]. In murine models that lack FGF-23 or klotho, the resultant hyperphosphatemia is associated with cell toxicity, premature aging and vascular calcifications [16].

Determining a relationship between phosphorus levels and prevalence of anemia in the non-CKD population may have important clinical implications. A study evaluating the National

30, 2016

RESEARCH ARTICLE

Serum Phosphorus Concentration and Coronary Artery Calcification in Subjects without Renal Dysfunction

Table 2. Odds ratios for Agatston score categories according to serum phosphorus concentrations.

Phosphorus (mg/dL)	Agatston score			
	0 < to ≤100		>100	
	OR (95% CI)*	P	OR (95% CI)*	p
Unadjusted				
≤3.2 (reference)	1.00		1.00	
3.2 < to ≤3.6	0.86 (0.66–1.12)	0.27	0.94 (0.66–1.36)	0.75
3.6 < to ≤4.0	0.94 (0.72–1.22)	0.66	1.17 (0.82–1.67)	0.39
>4.0	0.82 (0.62–1.09)	0.17	1.17 (0.81–1.70)	0.40
Case-mix adjusted[†]				
≤3.2 (reference)	1.00		1.00	
3.2 < to ≤3.6	0.90 (0.68–1.20)	0.48	1.09 (0.72–1.65)	0.69
3.6 < to ≤4.0	1.09 (0.82–1.45)	0.55	1.52 (1.01–2.30)	0.047
>4.0	1.14 (0.83–1.56)	0.41	2.01 (1.28–3.13)	0.002
Fully adjusted[‡]				
≤3.2 (reference)	1.00		1.00	
3.2 < to ≤3.6	0.88 (0.66–1.17)	0.39	1.10 (0.72–1.68)	0.45
3.6 < to ≤4.0	1.09 (0.82–1.45)	0.57	1.58 (1.04–2.40)	0.03
>4.0	1.10 (0.80–1.52)	0.55	2.11 (1.34–3.32)	0.001

* The multinomial logit model (baseline outcome category: Agatston score = 0) was used to calculate the odds ratio (OR) and 95% confidence interval (CI).

[†] The case-mix adjusted model included age, sex, diabetes and hypertension.

[‡] The fully adjusted model included all of covariates in the case-mix adjusted model and body mass index, systolic blood pressure, corrected serum calcium, albumin, hemoglobin A1c, LDL cholesterol and HDL cholesterol additionally.



Phosphorus as predictive factor for erectile dysfunction in middle aged men: A cross sectional study in Korea

Seung Ki Min¹, Kwibok Choi¹, Soon Ki Kim¹, Gyeong In Lee², In-Chang Cho¹Departments of ¹Urology and ²Laboratory Medicine, National Police Hospital, Seoul, Korea**Table 1.** Patients' characteristics according to IIEF-5 degree (n=1,899)

Variable	No or mild (17-25)	Mild to moderate (12-16)	Moderate to severe (≤ 11)	p-value
Age (y)	51.4 $\pm$ 4.8	52.8 $\pm$ 4.3	54.0 $\pm$ 4.0	<0.001
Body mass index (kg/m ²)	24.8 $\pm$ 2.4	25.1 $\pm$ 2.4	25.0 $\pm$ 2.6	0.151
Testosterone (ng/mL)	4.8 $\pm$ 1.4	4.6 $\pm$ 1.3	4.7 $\pm$ 1.4	0.088
PSA (ng/mL)	0.96 $\pm$ 0.95	0.96 $\pm$ 0.76	1.00 $\pm$ 1.28	0.726
Phosphorus (mg/dL)	3.40 $\pm$ 0.46	3.50 $\pm$ 0.44	3.71 $\pm$ 0.50	0.004
PV (cm ³)	23.9 $\pm$ 6.8	25.2 $\pm$ 7.0	24.4 $\pm$ 7.1	0.002
MetS	341 (31.4)	159 (34.0)	136 (39.4)	0.021

Values are presented as mean $\pm$ standard deviation or number (%).

IIEF-5, International Index of Erectile Function-5; PSA, prostate-specific antigen; PV, prostate volume; MetS, metabolic syndrome.

Phosphorus is necessary for several various biological roles in the signal transduction of cells and energy exchange of human body. About 80%–90% of phosphorus is founded in excretion and translocation between parts of the human body. The latter process is important during the fast changes of metabolism [1,2]. Impaired intestinal phosphate absorption,

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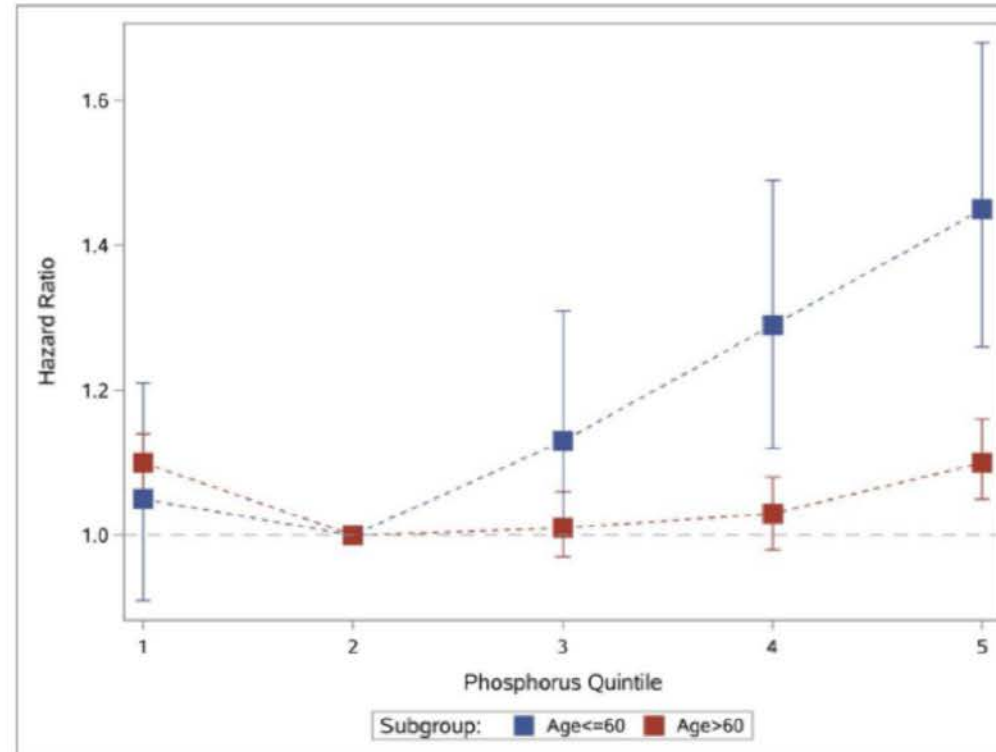
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RESEARCH ARTICLE

Serum phosphorus levels and risk of incident dementia

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Risk of Incident Dementia		Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
		Phosphorus ≤2.9 mg/dL	Phosphorus >2.9 and ≤3.2 mg/dL	Phosphorus >3.2 and ≤3.5 mg/dL	Phosphorus >3.5 and ≤3.9 mg/dL	Phosphorus >3.9 mg/dL
Age ≤ 60	N (%)	62,392 (21.90)	49,823 (17.49)	55,835 (19.60)	62,055 (21.78)	54,838 (19.25)
	HR(CI)	1.05 (0.91, 1.21)	1	1.13 (0.97, 1.31)	1.29 (1.12, 1.49)	1.45 (1.26, 1.68)
Age > 60	N (%)	112,038 (24.39)	93,071 (20.26)	96,278 (20.96)	92,801 (20.21)	65,104 (14.17)
	HR(CI)	1.10 (1.05, 1.14)	1	1.01 (0.97, 1.06)	1.03 (0.98, 1.08)	1.10 (1.05, 1.16)

HR, Hazard Ratio; CI, Confidence interval.

Model adjusted for age, race, gender, diabetes mellitus, cardiovascular disease, peripheral artery disease, hypertension, atrial fibrillation, depression, liver cirrhosis, baseline eGFR, number of eGFR measurements and number of hospitalizations.

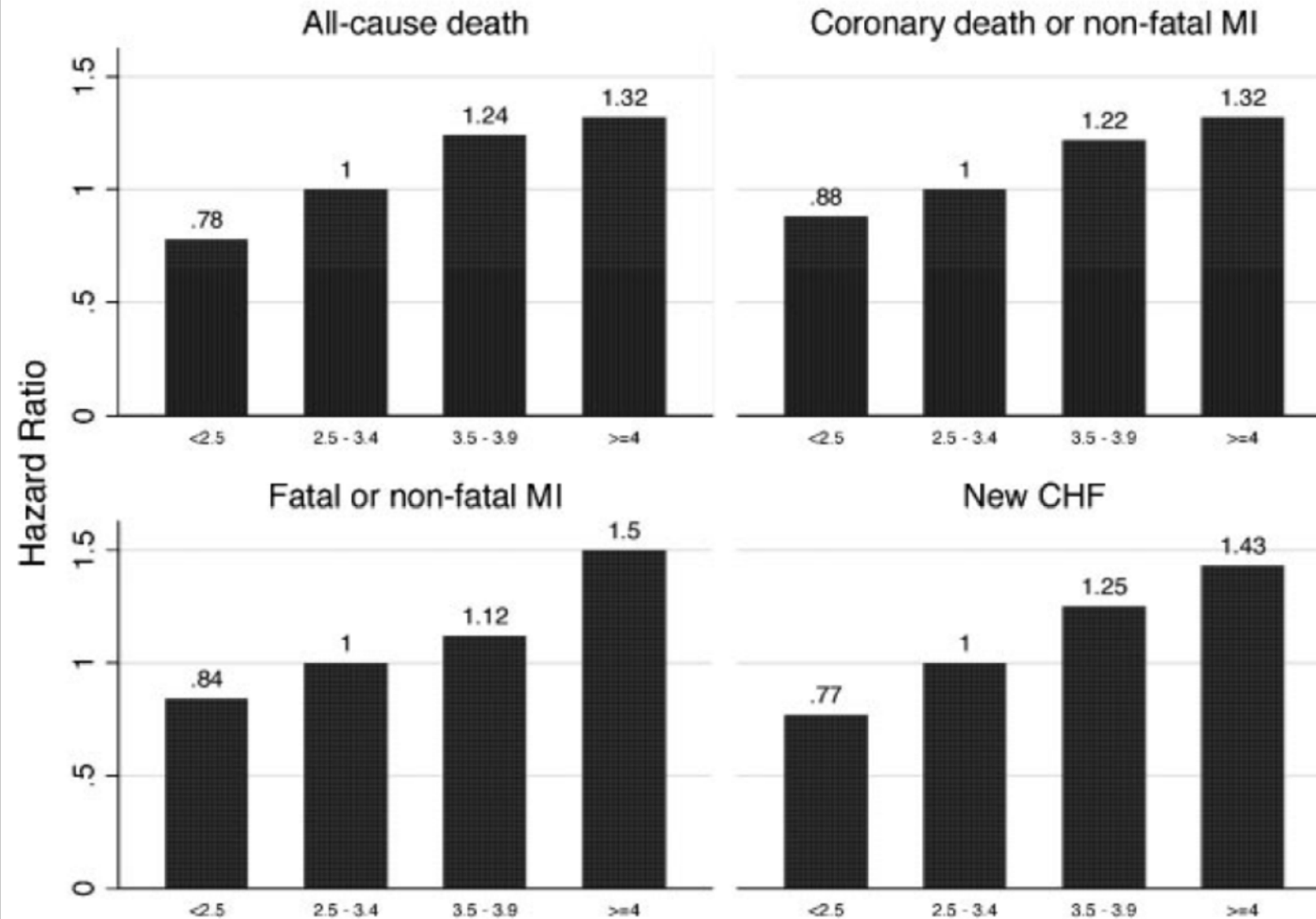
Quintile 2 (Phosphorus >2.9 and ≤3.2 mg/dl) served as the reference group.

* Indicates significant interaction ($p < 0.05$) between age group (≤ 60 , > 60) and serum phosphorus quintile.

Coronary Heart Disease

Relation Between Serum Phosphate Level and Cardiovascular Event Rate in People With Coronary Disease

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Phosphorus

- * Most phosphorus exists as phosphate in the body and is organically bound, but circulating inorganic phosphate is the form measured by a blood chemistry.

Traditional Reference range:

- * 2.5 – 4.5 mg/dL

Optimal Reference Range:

- * 3.1 – 3.5 mg/dL

Phosphorus - Elevated

Elevated – Functional hyperphosphatemia is due to either excessive phosphate intake, decreased phosphate excretion, or phosphate shift from intracellular to extracellular fluid.

Cause	Reason	Additional Inquiry
Vitamin D toxicity	Excess vitamin D increases phosphorus absorption from the intestines and increases phosphorus reabsorption in the kidneys.	Client history, vitamin D levels.
Hypoparathyroidism	PTH increases phosphorus excretion via the kidneys thus, low PTH encourages phosphorus reabsorption by the kidneys, thus increasing levels in the blood.	Evaluate calcium, PTH.
Kidney dysfunction	Poor phosphate filtering leads to increased levels in the blood.	Evaluate kidney markers.

The Prevalence of Phosphorus Containing Food Additives in Top Selling Foods in Grocery Stores

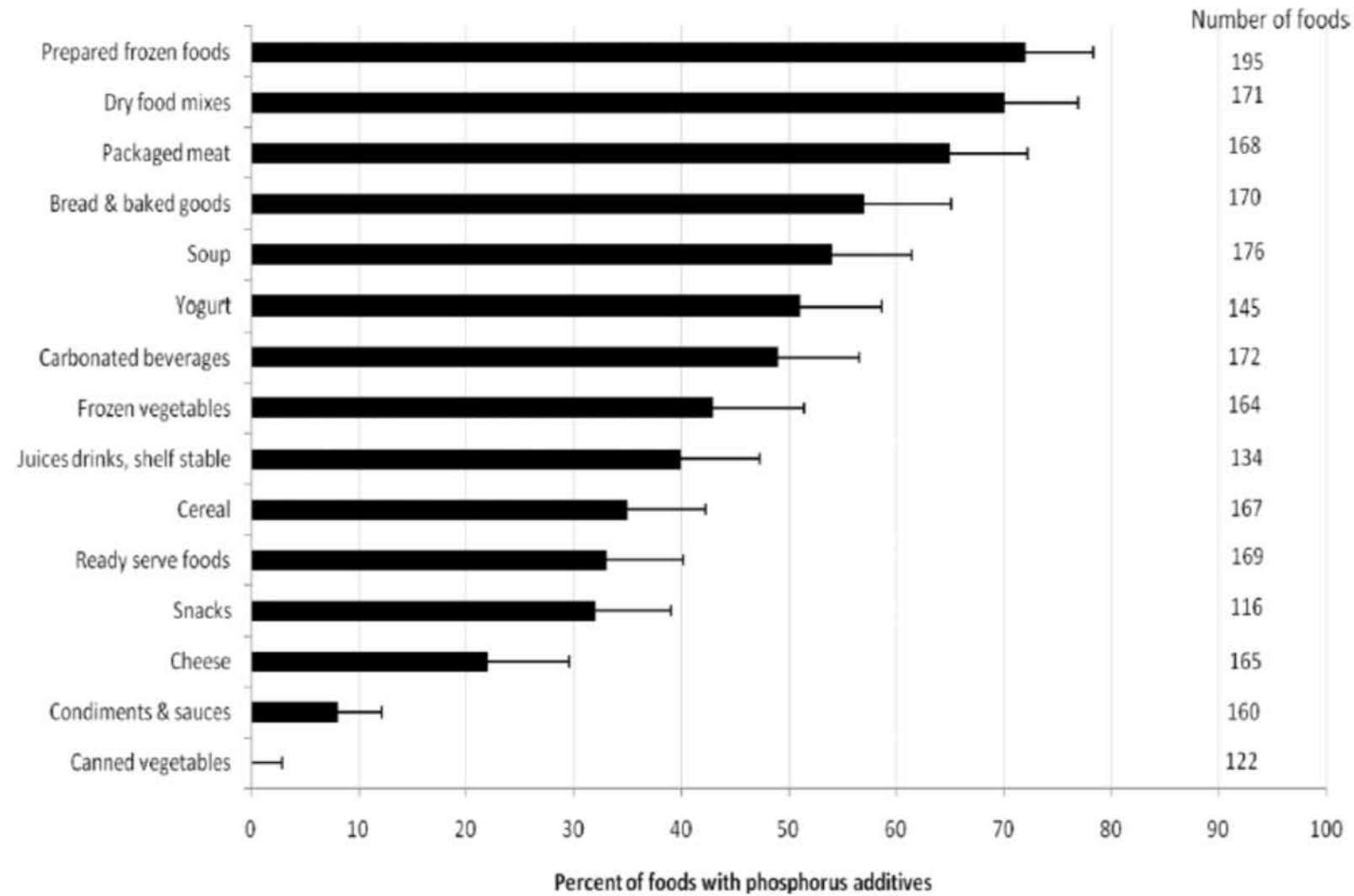


Figure 1. Prevalence of phosphorus containing food additives by food category (n=2394 total foods).

Phosphorus - Decreased

Cause	Reason	
Poor absorption	Phosphorus absorption can be inhibited by phytates, or excessive levels of magnesium, calcium, and aluminum.	Client history.
Vitamin D deficiency	Vitamin D stimulates phosphorus absorption from the intestines.	Evaluate vitamin D levels.
Increased insulin/high carb diet	Increased requirement for phosphorus intracellularly	
Diarrhea	Loss of phosphate.	
Poor protein digestion	Protein is a rich source of phosphorus of which 30-70% is absorbed. If an individual is consuming protein and phosphorus is low, it could be due to low hydrochloric acid and/or protein digestion and absorption. Additionally, hydrochloric acid requires hydrogen, chloride, and zinc.	

Superior predictive ability for death of a basic metabolic profile risk score

Table I. Standard ranges of normals for BMP parameters

Parameter	Range
Sodium (mmol/L)	
Normal	135-146
Low	<135
High	>146
Potassium (mmol/L)	
Normal	3.5-5.3
Low	<3.5
High	>5.3
Bicarbonate (mmol/L)	
Normal	20-32
Low	<20
High	>32
Calcium (mg/dL)	
Normal	8.5-10.3
Low	<8.5
High	>10.3
Glucose (mg/dL)	
Normal	<100
Intermediate	100-125
High	>125
Creatinine (mg/dL)	
Normal	0.8-1.19
Low	<0.8
High	1.2-1.99
Very high	>2.0

Superior predictive ability for death of a basic metabolic profile risk score

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