# Discussion on -

## Lipoprotein  metabolism
## &
## disorders

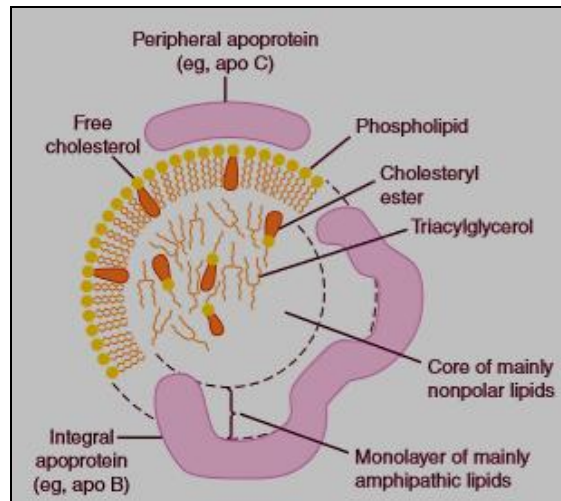**Presented by –**

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Dr. Rockshana Habib

Lecturer, Biochemistry

# Learning objectives

- **Definition & classification of lipoprotein**
- **Basic structure of lipoprotein**
- **Apolipoproteins**
- **Functions of lipoprotein**
- **Chylomicron metabolism & it's clinical aspects**
- **VLDL metabolism & it's disorders**

# Lipoprotein

# Lipoprotein

The **plasma lipoproteins** are the **spherical macromolecular complexes** of **lipid** and specific **proteins**.

# Classification of lipoprotein
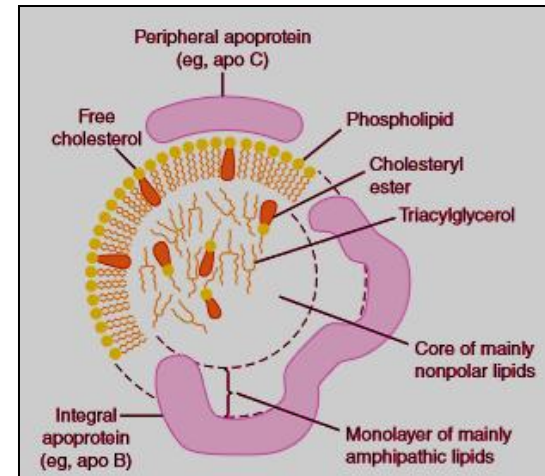
# Classification:

1.  Chylomicrons (CM)
2.  Very low density lipoproteins (VLDL)
3.  Low density lipoproteins (LDL)
4.  High density lipoproteins (HDL)
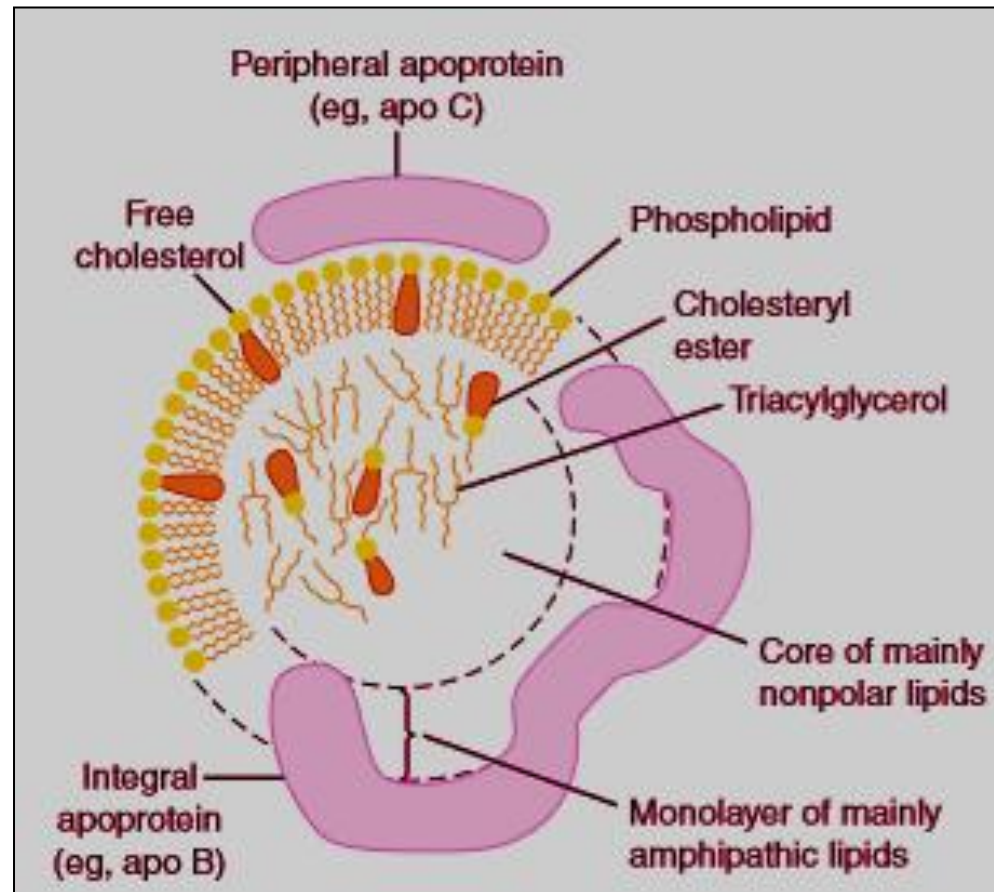
# **Structure of lipoprotein**

A **lipoprotein** basically consists of two parts –

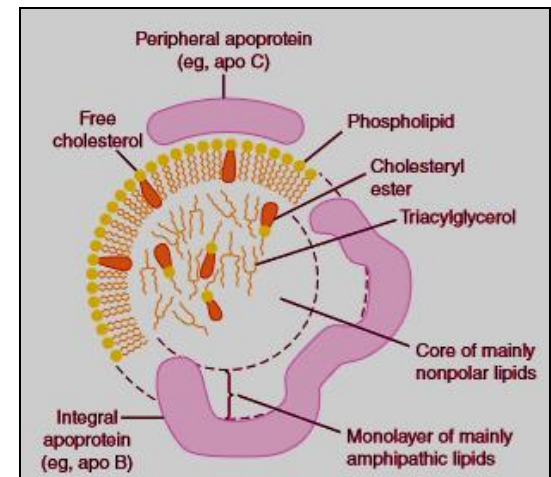1. A neutral **lipid core** and
2. A **shell**

# Structure of lipoprotein

# Apoprotein/Apolipoprotein

# Apoprotein/Apolipoprotein

The **protein component**
of **lipoproteins** is known as
**apoprotein**.



Peripheral apoprotein
(eg, apo C)

Free
cholesterol

Phospholipid

Cholesteryl
ester

Triacylglycerol

Core of mainly
nonpolar lipids

Integral
apoprotein
(eg, apo B)

Monolayer of mainly
amphipathic lipids

The **apoprotein** or **apolipoprotein**, constitutes

nearly **70%** of some **HDL** &

as low as

**1%** of **chylomicrones**.

# Apolipoprotein profile of lipoprotein:

| Lipoprotein | Inherent apoprotein | Acquired apoprotein |
|---|---|---|
| CM | B – 48 | C & E from HDL |
| VLDL | B - 100 | C & E from HDL |
| LDL | B - 100 | |
| HDL | A, C & E | |

# Functions of apolipoprotein

1. Act as a **structural  component** of **lipoprotein** to maintain their **structural stability**.

2. Act as **cofactor** for enzymes of lipoprotein metabolism.
   **e.g. Apo C-II** is a cofactor for **lipoprotein lipase (LPL).**

3. Act as **inhibitor** for enzymes of

lipoprotein metabolism.

   **e.g.** **Apo C-III** & **Apo A-II** inhibit

   **lipoprotein lipase**.

4. Act as **ligand** to recognize **lipoprotein receptors** on cell surface.

   **e.g. Apo B-100** & **Apo E** act as

   ligand for **LDL receptor**.

5.   Provide  **hydrophilic  character**  of  <span style="color:red">**lipoprotein particles**</span> to facilitate  their  transport  in  **aquous plasma**.

6.   Facilitate  exchange  of **lipids**  between  **lipoproteins**.

# Composition of Lipoproteins

| Lipoprotein | Source | Composition (%) | | Main lipid component | Apoprotein |
|---|---|---|---|---|---|
| | | Protein | Lipid | | |
| **Chilomicrons** | Intestine | 1-2 | 98-99 | TAG | A- I, A- II ,A- IV<br>B- 48<br>C- I,II,III, E |
| **VLDL** | Liver (Intestine) | 7-10 | 90-93 | TAG | B -100<br>C- I, II, III |
| **LDL** | VLDL | 21 | 79 | Cholesterol | B- 100 |
| **HDL** | Liver intestine, VLDL | 32 | 68 | Phospholipid, Cholesterol | A- I, II, IV<br>C- I, II, III<br>D, E |

# Functions of lipoprotein

# Chylomicrones:

1. Transport of **dietary lipids** from **intestine** to **liver** & other **peripheral tissues**.
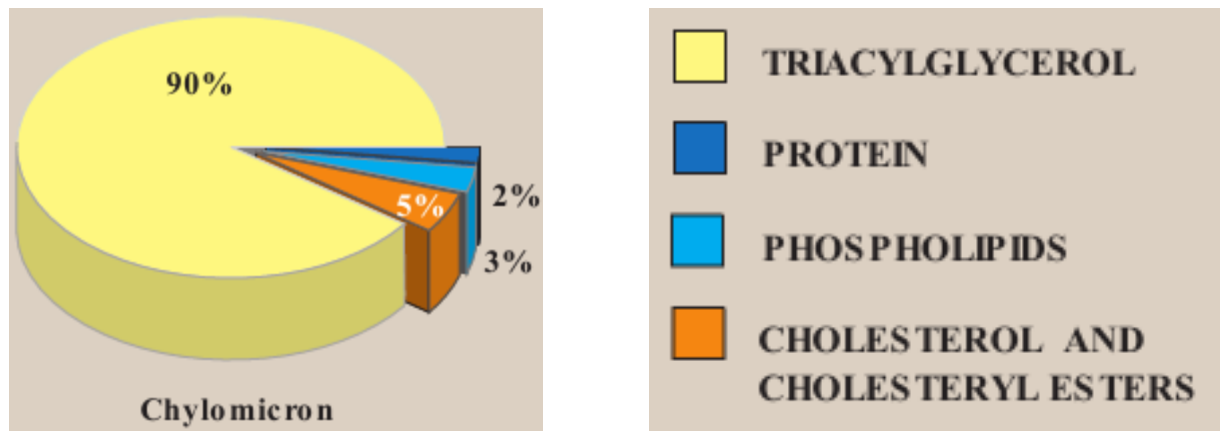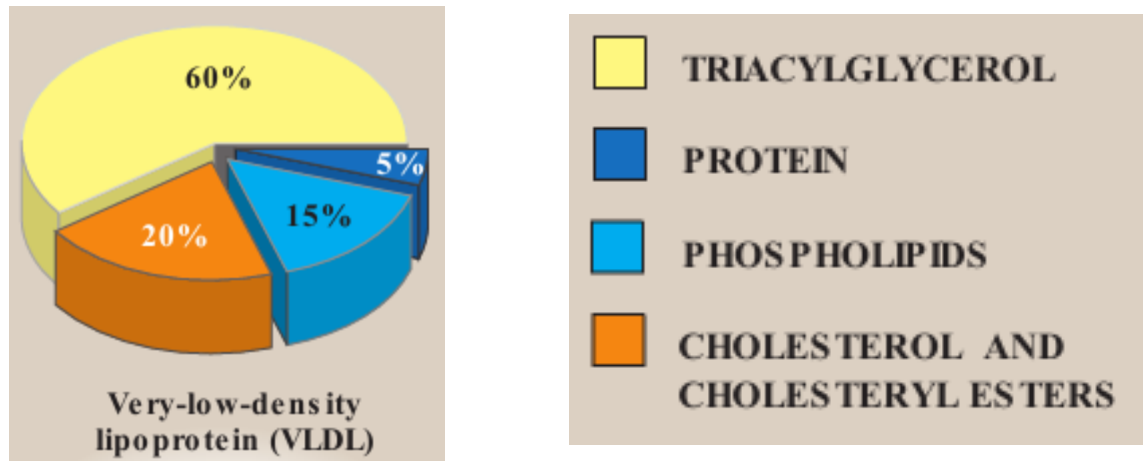
# Very low density lipoprotein:

1. Supports **endogenous lipid transport**.
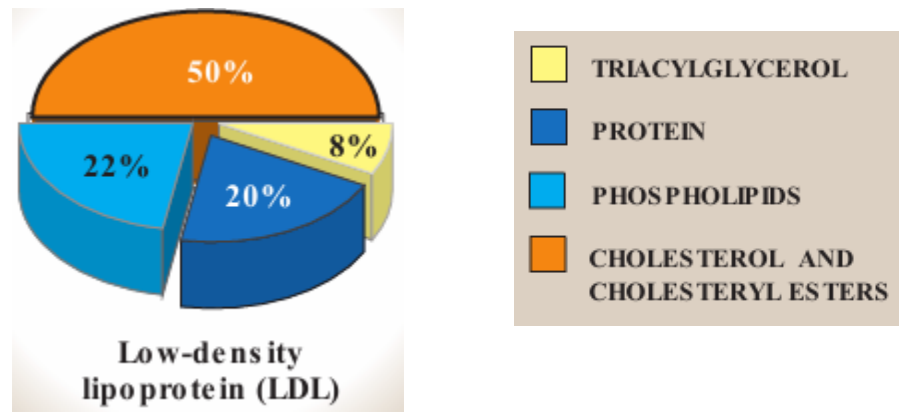
2. Carries **TAG** from **liver** to **peripheral tissues**.

3. Carries **cholesterol** via **LDL** from **liver** to **peripheral tissues**.

4. Acts as **precursor** for **LDL**.



60%

5%

20%

15%

Very-low-density lipoprotein (VLDL)

TRIACYLGLYCEROL

PROTEIN

PHOSPHOLIPIDS

CHOLESTEROL AND CHOLESTERYL ESTERS

## Low density lipoprotein:

1. Supports **endogenous lipid transport**.

2. Receives **cholesterol** from **VLDL** & **HDL** and then carries to the **peripheral tissues**.



50%

22%

20%

8%

Low-density lipoprotein (LDL)

TRIACYLGLYCEROL

PROTEIN

PHOSPHOLIPIDS

CHOLESTEROL AND CHOLESTERYL ESTERS
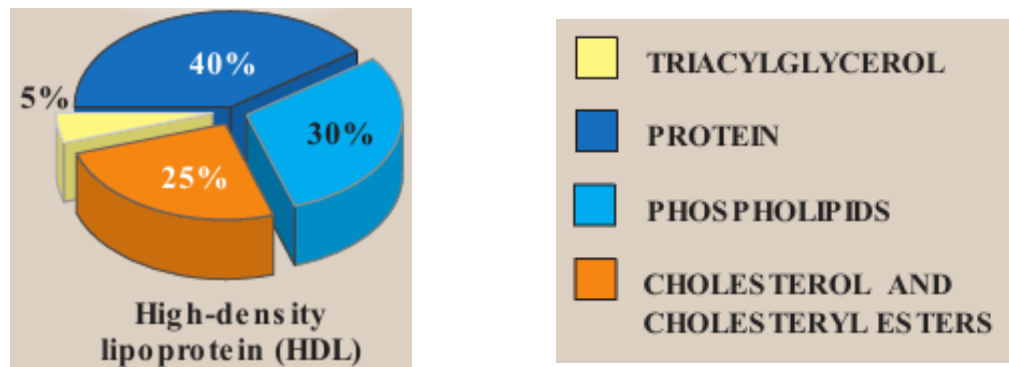
## High density lipoprotein:

**1.** Supports **reverse cholesterol transport** to keep **serum cholesterol** normal .

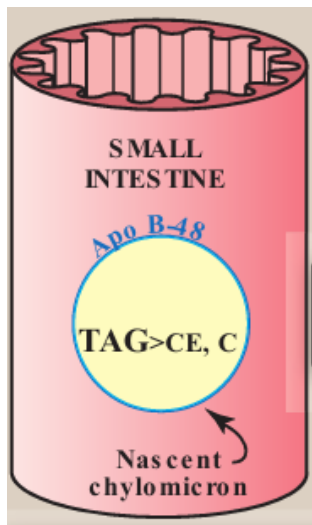**2.** Carries **cholesterol** from **peripheral tissues** back to the **liver** for **biliary excretion**.

**3.** It has native **antioxidant activity**.

**4.** Supports **steroidogenesis** by providing **cholesterol**.

**5.** Helps in **chylomicrone** & **VLDL** metabolism by providing **apo C II** & **apo-E.**
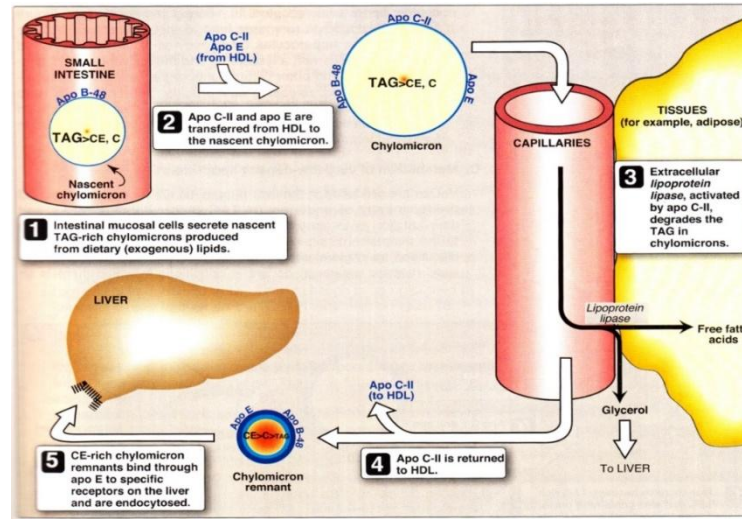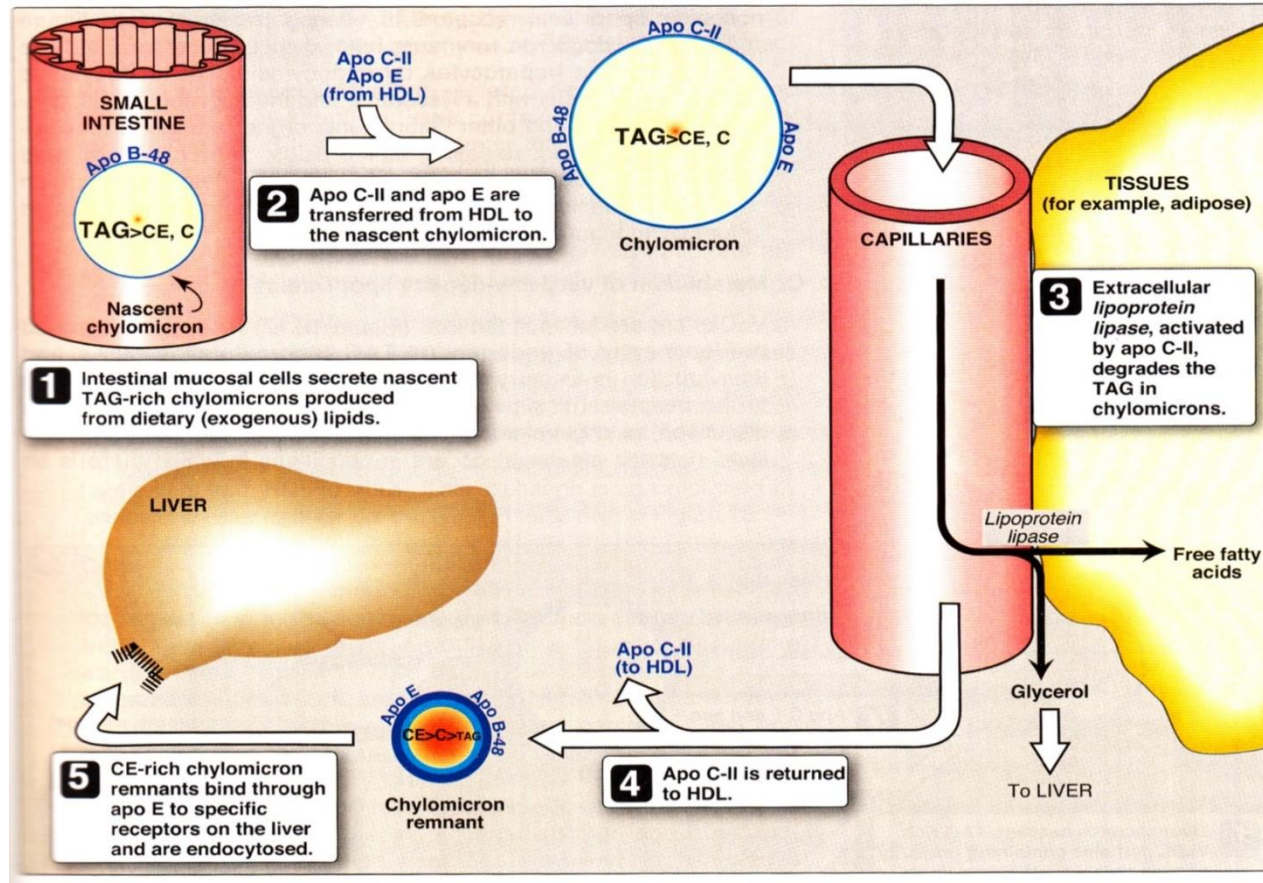
# Metabolism of chylomicrones

# Metabolism of chylomicrones:

**Steps:**

1. Synthesis of **Apo B-48** in intestinal epithelial cell.

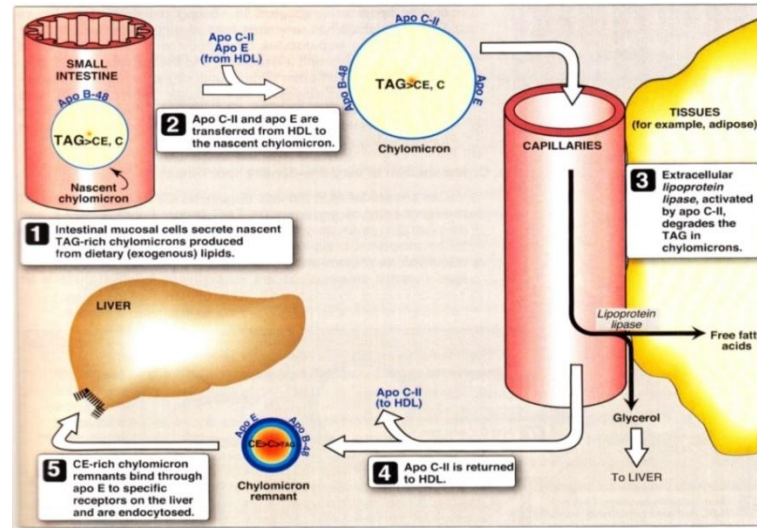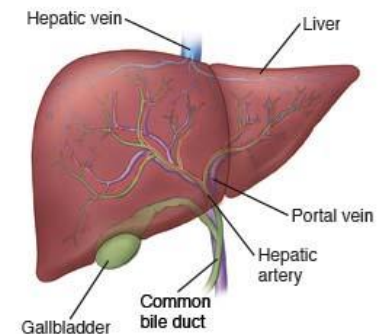2. Synthesis & release of **nascent chylomicron (CM).**

# Metabolism of chylomicrons:

# 3. Modification of nascent chylomicron in plasma by receiving apo C- II & apo E from HDL.

# 4. Degradation of  TAG by lipoprotein  lipase

The **<span style="color:red">fatty acids</span>** are stored in **<span style="color:purple">adipose tissue</span>** & **glycerol** is used by the **<span style="color:blue">liver</span>**.

Hepatic vein

Liver

Portal vein

Hepatic artery

Common bile duct

Gallbladder

The size of the **mature CM**

after loosing **TAG**

decreases

by **75%**.

**Apo C-II** is returned back to

**HDL** &

**CM** turns into

**chylomicron remnant**

rich in

**cholesterol.**

# 5. Clearance of  chylomicron remnant by the liver:
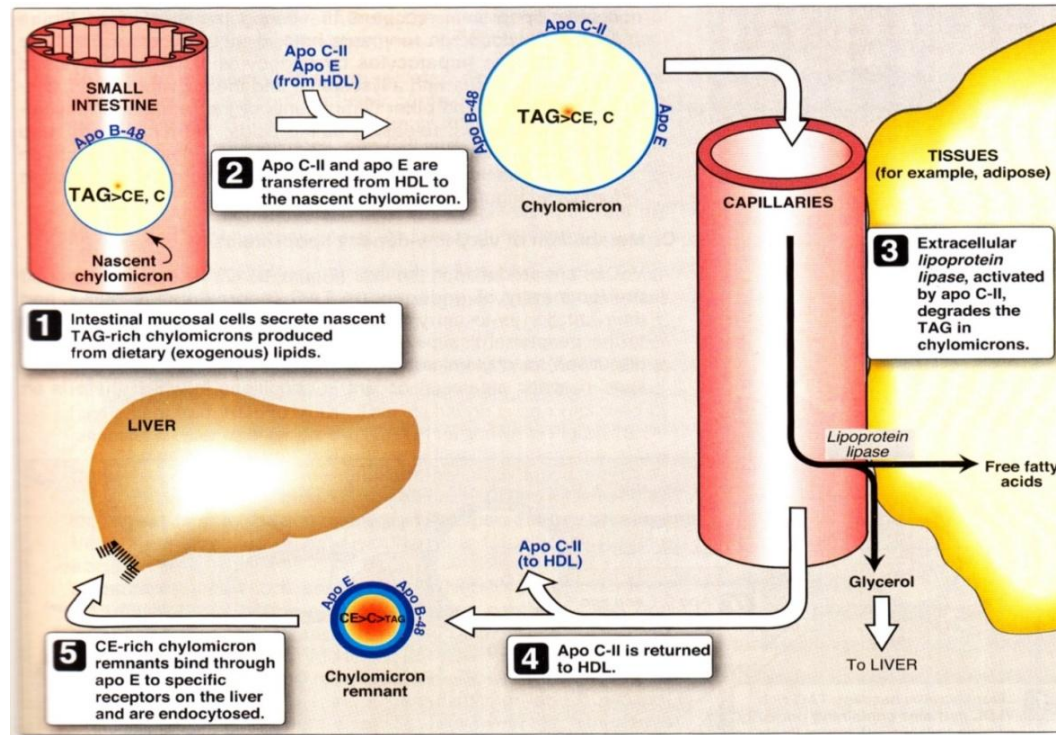
# Clinical aspects of chylomicron metabolism

# Clinical aspects of chylomicron metabolism:

Patients with a **deficiency** of
**LPL** or **apo C-II**
suffer from

**type 1 hyperlipoproteinemia**, or
**familial LPL-deficiency**
**and …**

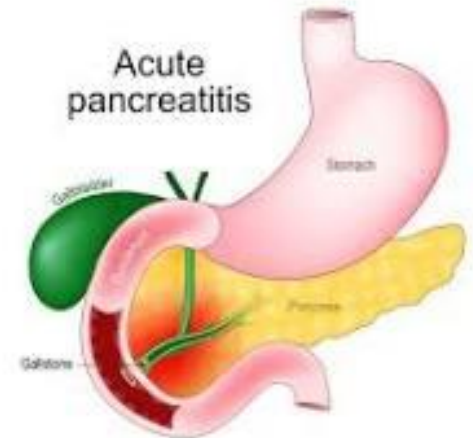**Clinical aspects of chylomicron metabolism…cont.**

show a dramatic **accumulation**

**(1,000 mg/dl or greater)** of

**chylomicron-TAG**

in the **plasma**

**(hypertriacylglycerolemia)**

even in the **fasted state.**



**Lipemia**

# Clinical aspects of chylomicron metabolism…cont.

These individuals

are at **increased risk**

for

**acute pancreatitis**.



Acute
pancreatitis

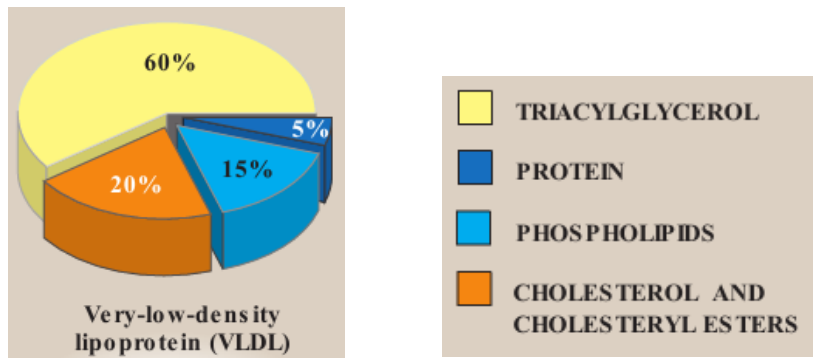# Metabolism of VLDL

# Metabolism of VLDL:

**VLDLs** are produced in the **liver.**

They are composed predominantly of **TAG** **(60%).**



**Very-low-density lipoprotein (VLDL)**

60%
5%
15%
20%

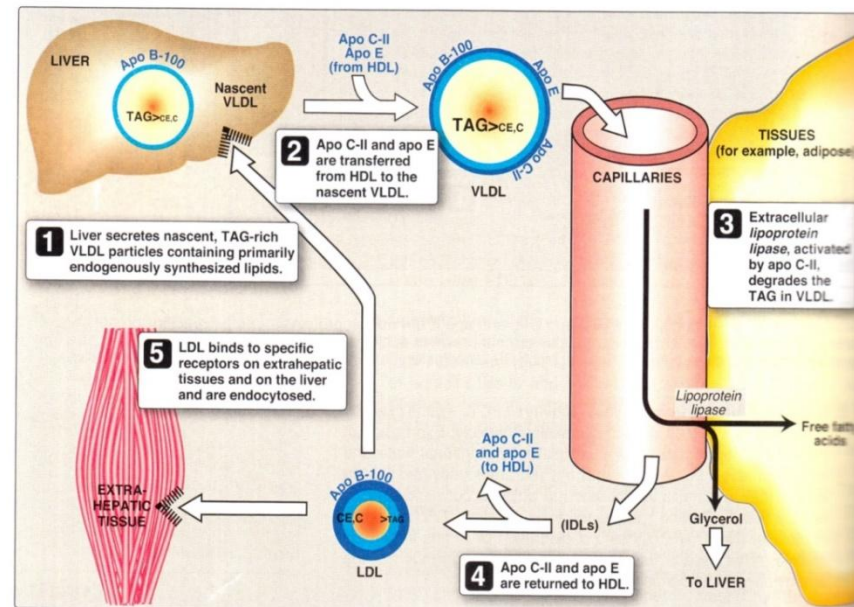- TRIACYLGLYCEROL
- PROTEIN
- PHOSPHOLIPIDS
- CHOLESTEROL AND CHOLESTERYL ESTERS

They carry **TAG**
from the **liver** to
**peripheral tissues**.

The **TAG** then degraded by
**lipoprotein lipase (LPL)**.

**Steps:**

**1. Synthesis of apo B-100 in liver**

**2. Release of  nascent  VLDL**

# 3. Modification of nascent VLDL in plasma
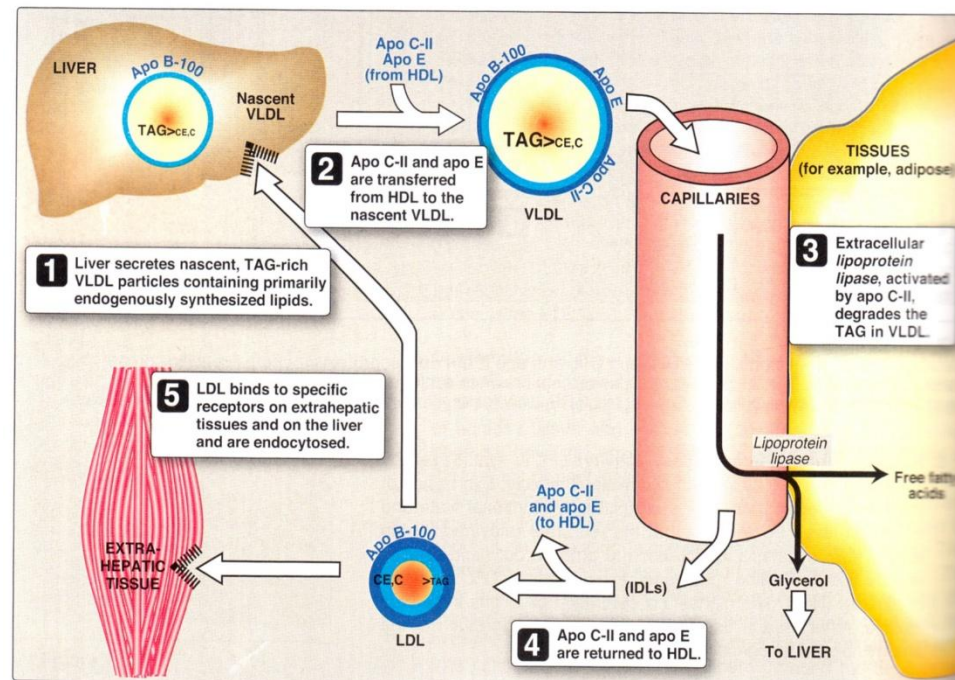
# 4. Exchange of lipid with HDL:

**VLDL** receives cholesteryl esters

from **HDL** &

in exchange gives

**TAG** & **free cholesterol** to

**HDL**.

# 5. Production of LDL from VLDL in the plasma:

With this modification,

the **VLDL** is converted

in the plasma to

**LDL**.
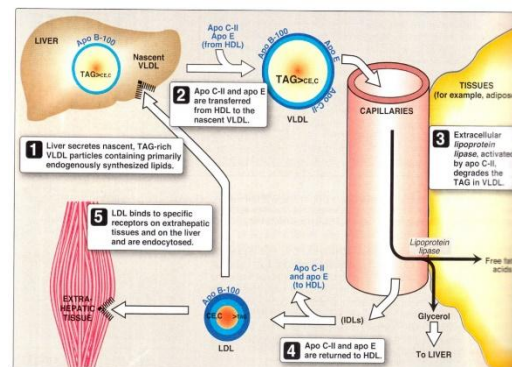
# **Disorder of lipoprotein (VLDL) metabolism**

## **Fatty liver/ Hepatic steatosis**

Healthy liver   Fatty liver disease   Hepatic cirrhosis
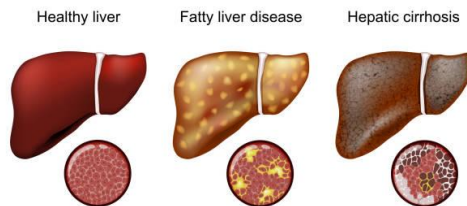
# **Fatty liver/ Hepatic steatosis**:

It is a clinical condition in which there is an **imbalance** between hepatic **TAG synthesis** & the **secretion** of **VLDL** occurs in -

    a.  **Obesity**

    b.  Uncontrolled **diabetes mellitus**

    c.  Chronic **ethanol ingestion**

**Fatty liver disease** is of two types –

**i.** Alcoholic fatty liver disease

**ii.** Non-alcoholic fatty liver disease (NAFLD)

# Non-alcoholic fatty liver disease ( NAFLD )

# Non-alcoholic fatty liver disease

Increasingly **sedentary lifestyles** and
changing **dietary patterns**
mean that
the prevalence of **obesity** and
**insulin resistance**
has increased worldwide.

In the **absence** of

**high alcohol consumption**

this  is called

**non-alcoholic fatty liver disease (NAFLD)**.

**Fat** accumulation in the **liver**

is a **common finding**

during **abdominal imaging** studies

and

on **liver biopsy**.

# **NAFLD** includes

# a **spectrum** of

# **progressive** **liver disease**.

# **NAFLD** is considered to be the **hepatic manifestation** of the '**Metabolic syndrome**'
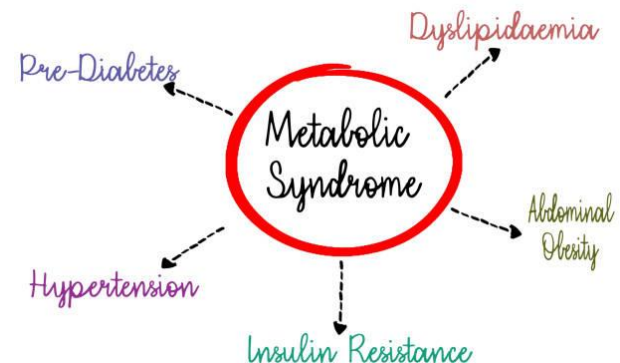
# Prevalence of NAFLD

# Prevalence of NAFLD



Age-standardised deaths per 100 000 people
- ≥8·4
- 5·6 to <8·4
- 4·3 to <5·6
- 3·3 to <4·3
- <3·3
- Not applicable
- No data
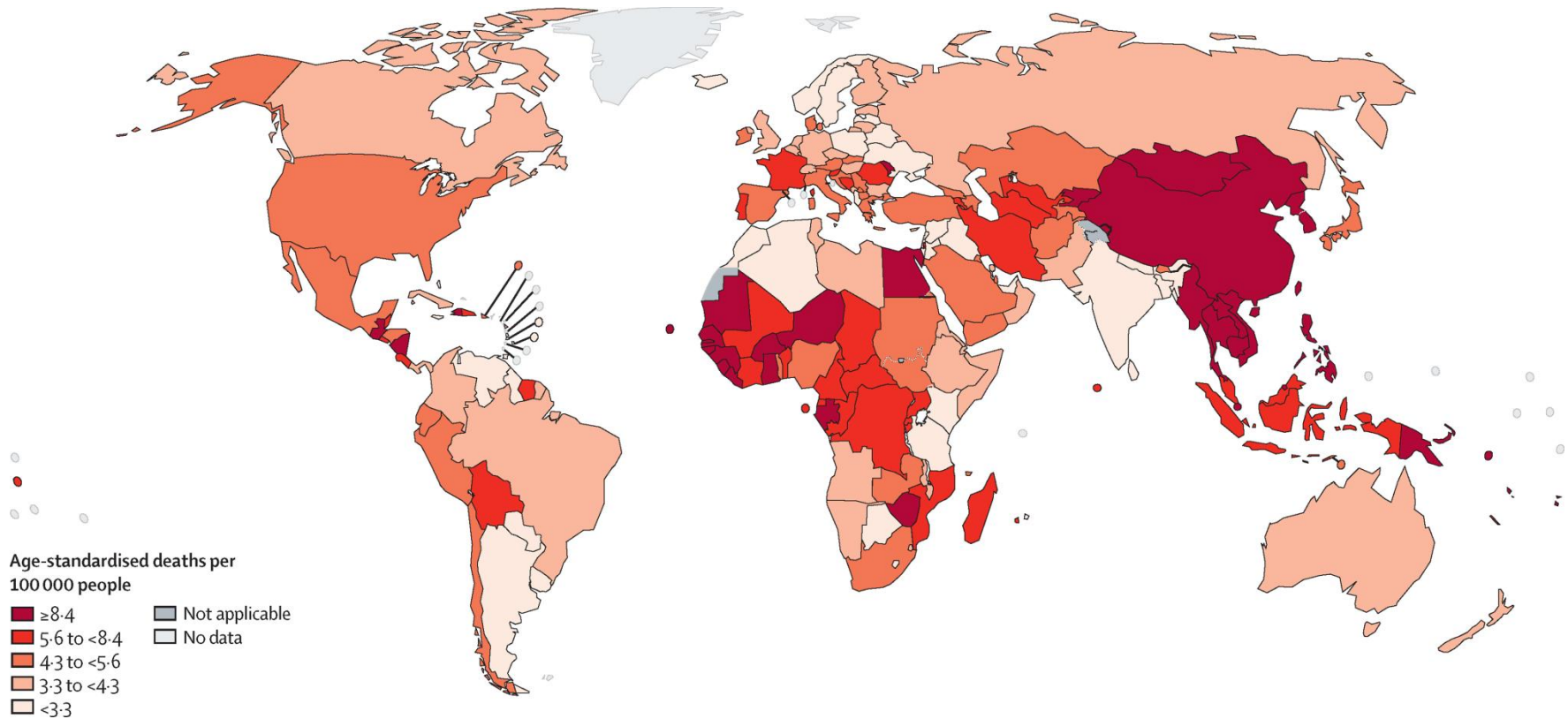
# Prevalence of NAFLD
## Bangladesh perspective
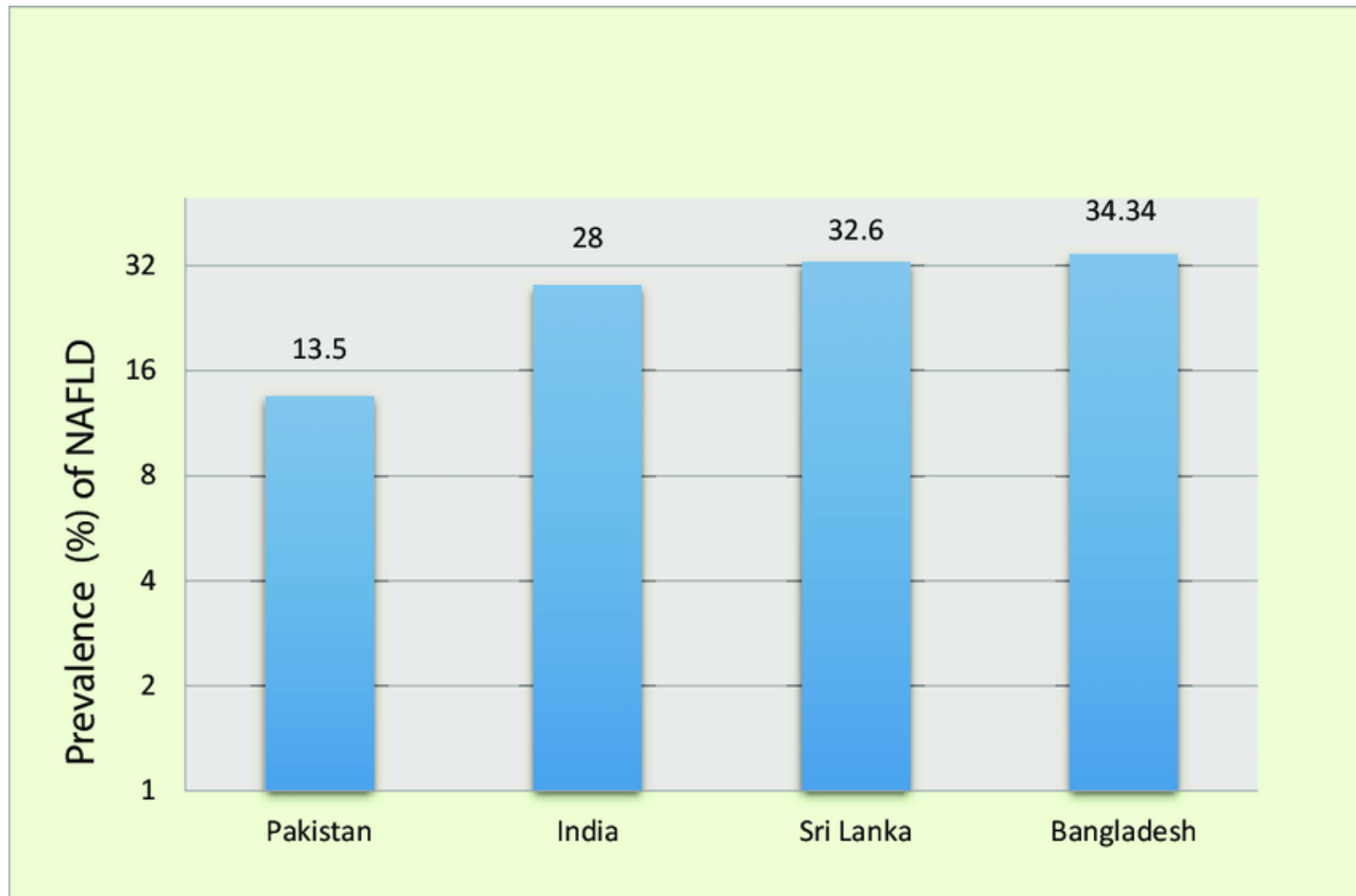
# Disease prevalence

Estimates **vary**

**between populations**,

although one **large European study**

found

**NAFLD**

to be present in

**94%**.

The overall **prevalence** of

**NAFLD**

in patients with

**type 2 diabetes**

ranges from

**40% to 70%**.

The frequency of **steatosis** varies with ethnicity-

- 45% in **Hispanics**
- 33% in **whites**
- 24% in **blacks**

but only a **minority** of patients

will progress to

**Cirrhosis**

and

**end-stage liver disease**

Over a median **12-year follow-up** period

in a **cohort** of **619 NAFLD** patients -

an overall **33.2%** risk of

**death** or

**liver transplantation**

was observed,

**with ...**

**liver-related mortality**

being the **third** most

common cause of **death**

after

**cardiovascular disease** and

**extra-hepatic malignancy**.

**NAFLD**

is the **leading cause** of

**liver dysfunction**

in the

**non-alcoholic.**

# Spectrum of NAFLD

# Spectrum of NAFLD

**NAFLD** ranges from –

- **fatty infiltration** alone - **(steatosis)**

To

- **fatty infiltration** with **inflammation**

  **(non-alcoholic steatohepatitis, NASH)**

**and …**

may **progress** to

**cirrhosis**

and

**primary liver cancer**.

# Spectrum of NAFLD
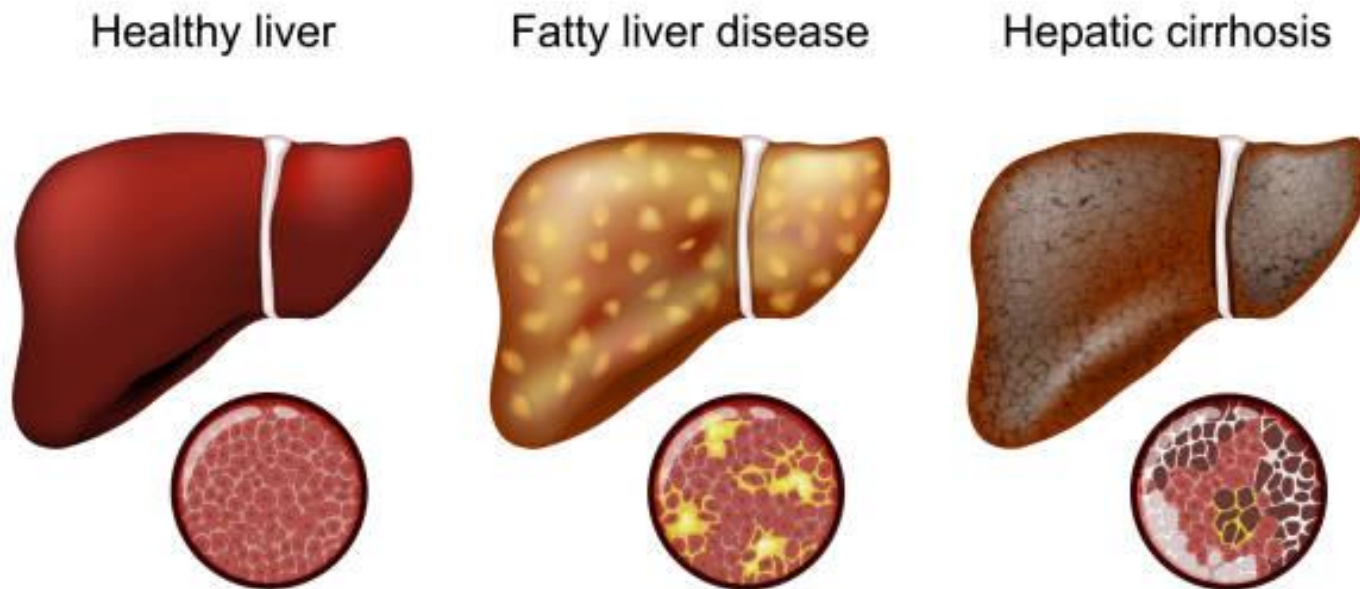


Healthy liver     Fatty liver disease     Hepatic cirrhosis

# Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH)



A

Steatosis → NASH → Cirrhosis

↑FA influx
↓FA oxidation
↑FA synthesis
↓VLDL assembly
Insulin resistance

TNF-α
Oxidant stress
Endotoxin
Immune factors

TGF-β
Stellate cell activation

Fat infiltration > 5% with or without mild inflammation

Steatosis + necroinflammation (ballooning, Mallory bodies, megamitochondria)

Increasing fibrosis, eventually leading to cirrhosis
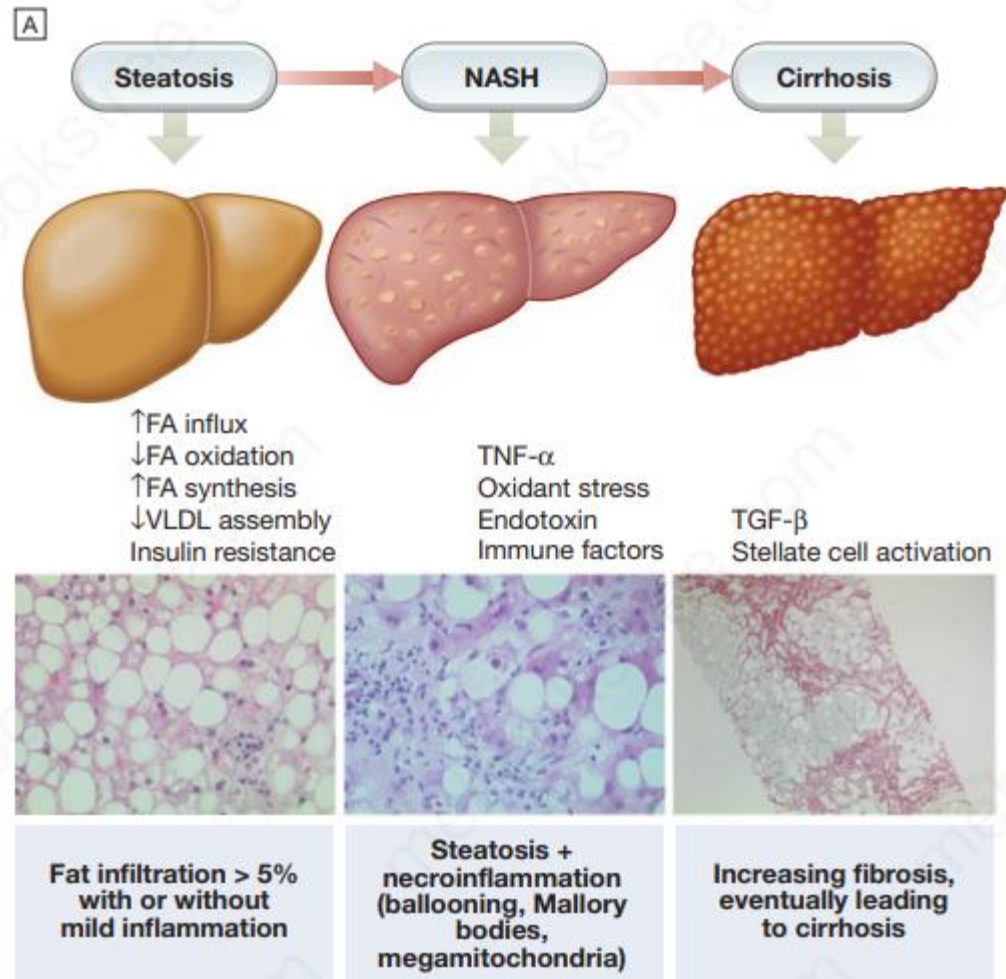
(FA = fatty acid; TGF-β = transforming growth factor beta;
TNF-α = tumour necrosis factor alpha; VLDL = very low-density lipoprotein)

# The spectrum of NAFLD



B
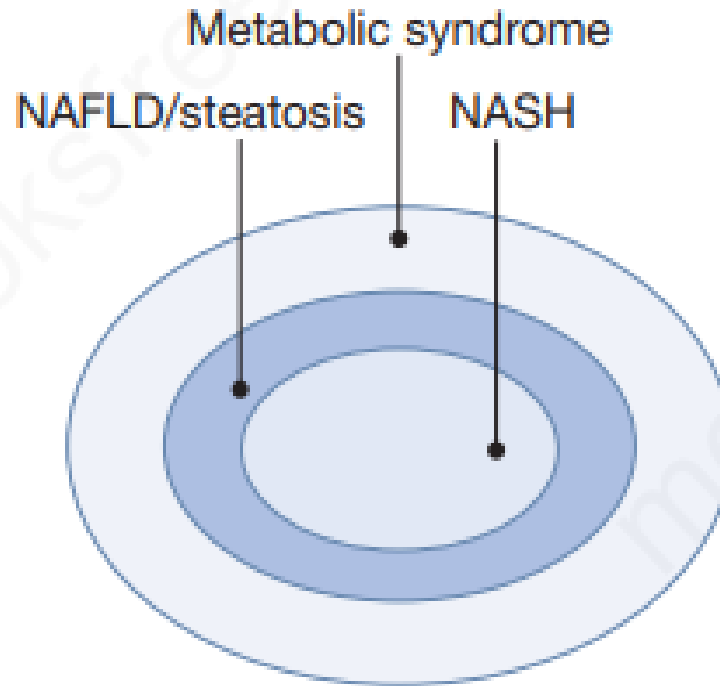
Metabolic syndrome

NAFLD/steatosis          NASH

**Non-alcoholic fatty liver disease (NAFLD) and
non-alcoholic steatohepatitis (NASH)**

The **histological** definition of **NASH**
is based on a
combination of **three lesions-**

- **steatosis**,
- **hepatocellular injury** and
- **inflammation**

NASH  it is **strongly associated** with –

- **obesity,**
- **dyslipidaemia,**
- **type 2 diabetes and**
- **hypertension.**

NAFLD is also associated with –

- **Polycystic ovary syndrome**
- **Obstructive sleep apnea** and
- **Small-bowel bacterial overgrowth**

# Risk factors



Obesity, Inactive, Genetics, Aging, Medications, Rare disorders → Insulin Resistance → Type 2 diabetes, PCOS, Atherosclerosis, Dyslipidemia, Hypertension
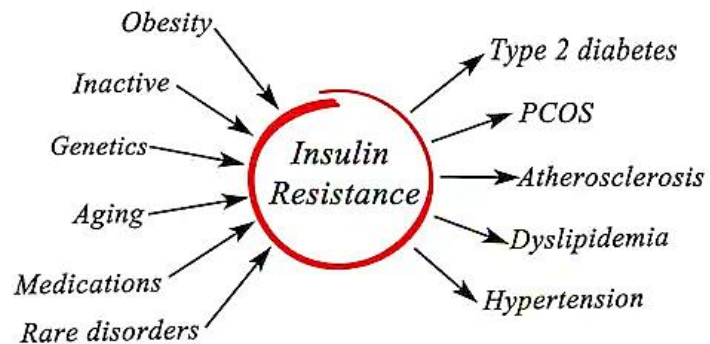
The emerging **epidemic** of

**childhood obesity**

means that

**NASH**

is present in increasing numbers of

**younger patients**.

**Risk factors…cont.**

**Age group:**

**40–50 years:** The average age of **NASH**

patients

**50–60 years:** for **NASH–cirrhosis**

Recognized **independent** **risk factors** for **disease progression** are-

- **age** over **45 years**,
- presence of **diabetes**
  (or severity of **insulin resistance**),
- **obesity** (BMI >30 kg/m$^2$ ) and
- **hypertension**.

These factors

help with **identification** of

**'high-risk'**

patient groups.

# Clinical features

**Clinical features of NAFLD:**

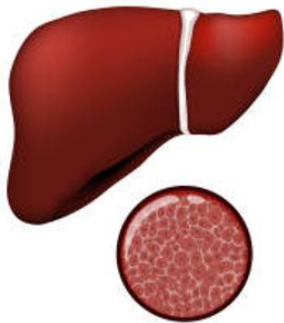1. Frequently **asymptomatic**

2. **Fatigue**

**Clinical features of NAFLD:**

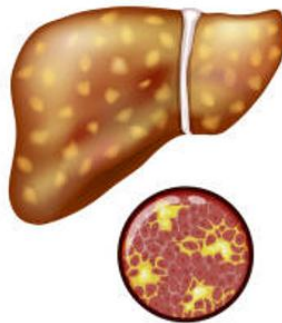**3.** Mild **right upper quadrant discomfort**.

# Pathophysiology



Healthy liver    Fatty liver disease

# Pathophysiology

The **initiating events** in
**NAFLD** are
typically based on the **development** of
**obesity** and **insulin resistance**,
leading to **increased**
**hepatic**
**free fatty acid** influx.

This **imbalance** between

the **rate of import/synthesis**

and

the **rate of export/catabolism** of

<span style="color:purple">**fatty acids**</span>

in the <span style="color:green">**liver**</span>

leads to the development of

<span style="color:blue">**steatosis**</span>.



Healthy liver    Fatty liver disease    Hepatic cirrhosis
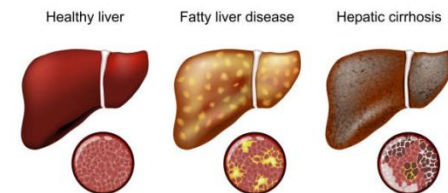
This may be an
**adaptive response**
through which
**hepatocytes** store potentialy
**toxic lipids**
as relatively **inert**
**triglyceride**.

A **'two-hit'** hypothesis

has been **proposed**

to describe

the **pathogenesis** of

**NAFLD**.

Healthy liver    Fatty liver disease    Hepatic cirrhosis
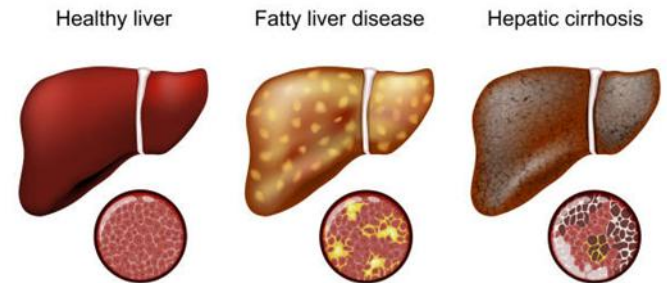
the **'first hit'** causing

**steatosis**

that then **progresses**

to

**NASH** or **fibrosis**

if a **'second hit'** occurs.

In reality,

**progression** probably **follows**

**hepatocelular injury**

caused by a

**combination** of

**several different 'hits'**,

Including …

- **oxidative stress** due to **free radicals** produced during **fatty acid oxidation**

- direct **lipotoxicity** from **fatty acids** and other **metabolites** in the **liver** .

- **endoplasmic reticulum** stress

- gut-derived **endotoxin**

- **cytokine** release **(TNF-α)** and **immune-mediated hepatocellular injury.**

**Cellular damage**
triggers
**cell death** and **inflammation**,
which leads to
**stellate cell** activation and
development of **hepatic fibrosis** that
**culminates** in
**cirrhosis**.

As with many other **liver diseases**, **genetic** and **environmental factors** **interact** to determine **disease progression …**

## a. Genetics:

Several genetic modifiers of disease severity-

**PNPLA3**   and it's product **adiponutrin**.

**(PNPLA3 gene:**

**Patatin like phospholipase domain containing protein 3)**

## b. Demographics:

- **Cirrhosis** is more common with increasing **age**, **duration of disease**.

- Higher rates of **fibrosis progression** are observed in **men** and **post-meno pausal women**.

## c. Diet:

- Excessive **alcohol consumption** leads to increased **fibrosis** progression.

- **Coffee consumption** appears to be **protective** against **fibrosis** and **HCC** in **NAFLD**.
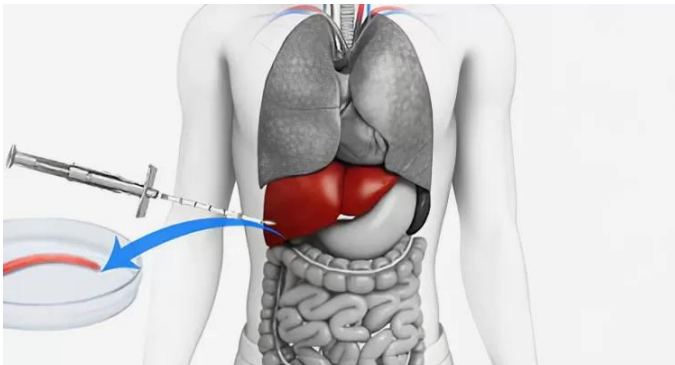
## D. Intestinal microbiota:

**Obesity**, **diabetes** and **NAFLD** are all linked with **gut dysbiosis**, with several **microbial infections** associated with advanced **liver inflammation** and **fibrosis**.
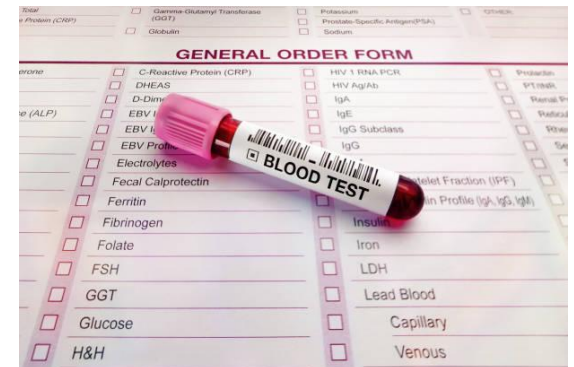
**e. Comorbidity:**

The presence of **type 2 diabetes** and severe **obesity** are associated with higher rates of **cirrhosis** in **NAFLD**.

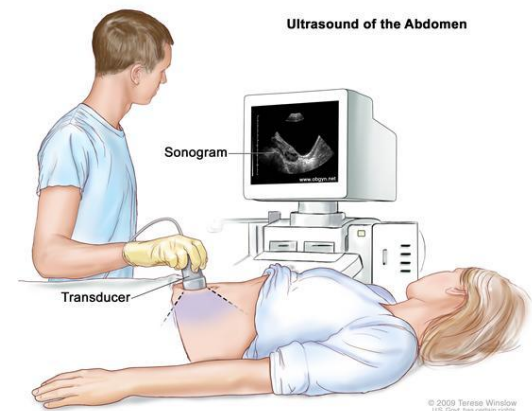# Diagnosis of NAFLD

**NFALD** is commonly identified

as an **incidental**

**biochemical abnormality**

during

**routine blood tests**

**or …**

as a **fatty liver**

during an **ultrasound** or

**CT scan**

of the **abdomen**.



Ultrasound of the Abdomen

Sonogram

Transducer

© 2009 Terese Winslow
U.S. Govt. has certain rights

**Alternatively,**

patients with **<span style="color:purple">progressive NASH</span>**

may present **<span style="color:red">late</span>**

in the **natural history** of the disease with
**<span style="color:green">complications</span>** of

**<span style="color:blue">Cirrhosis</span>**

**and …**

Cirrhosis

**portal hypertension**,

such as

**variceal haemorrhage**,

or **with**

**hepatocellular carcinoma**.
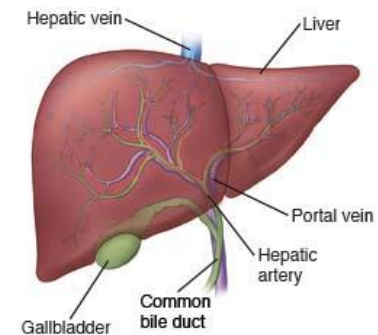
# Investigations of NAFLD

**Investigations** of patients with
**suspected NAFLD**
should aim to **confirm**
the presence of **fat**
in the **liver** and
**determine** the extent of
**fibrosis**.

**Investigations**

should **exclude**

other **coexistent liver diseases**

including

**viral**, **autoimmune** & **inherited causes.**

**1. Biochemical tests:**

There is **no**

**single**

diagnostic **blood test**

for

**NAFLD**.

- **Serum glucose**, **serum lipid profile**.

- **Serum GGT** is often **raised**.

- **ALT** and **AST** may be **normal** or **modestly raised**  usually **less** than **twice** the upper limit of normal.

**ALT** levels **fall** as

**hepatic fibrosis** **increases**

and the normal

**AST : ALT ratio** of **< 1**

**reverses**

as advanced **fibrosis develops**.

**Other laboratory abnormalities –**

**low-titre antinuclear antibody** (ANA)

in **20%–30%** of patients

and

**elevated ferritin levels**.

# Although
# routine blood tests
# are
# unable to determine
# the degree of
# liver fibrosis.

**NAFLD Fibrosis Score** and **FIB-4 Score**

which are based on

the results of

routinely available **blood tests**

and

**Anthropometrics** variable.

# Formula for calculating fibrosis scoring

- **NFS =** -1.675 + (0.037 * age) + (0.094 * BMI) + (1.13 * diabetes (yes=1, no=0)) + (0.99 * AST/ALT ratio) - (0.013 * platelet count) - (0.66 * albumin)

- **FIB-4 =** [Age (years) x AST (U/L)) / Platelet Count (109/L) x √(ALT (U/L)]

| | | Thresholds | |
|---|---|---|---|
| **Test** | **Key variables** | **Age < 65 yr** | **Age > 65 yr** |
| **NAFLD Fibrosis Score (NFS)[2]** | Age<br>BMI<br>Diabetes/IFG<br>AST<br>ALT<br>Platelets<br>Albumin | **High risk**<br>>0.676<br>**Indeterminate risk**<br>−1.455–0.676<br>**Low risk**<br><−1.455 | **High risk**<br>>0.676<br>**Indeterminate risk**<br>0.12–0.676<br>**Low risk**<br><0.12 |
| **FIB-4 Score[3]** | Age<br>AST<br>Platelets<br>ALT | **High risk**<br>>2.67<br>**Indeterminate risk**<br>1.30–2.67<br>**Low risk**<br><1.30 | **High risk**<br>>2.67<br>**Indeterminate risk**<br>2.00–2.67<br>**Low risk**<br><2.00 |

**24.50 Simple non-invasive scores for NAFLD/fibrosis[1]**

**113**

These scoring systems
have a
**high negative predictive value**
for **advanced**
**fibrosis/cirrhosis.**

# Progressive liver fibrosis assessed by Metavir scoring system
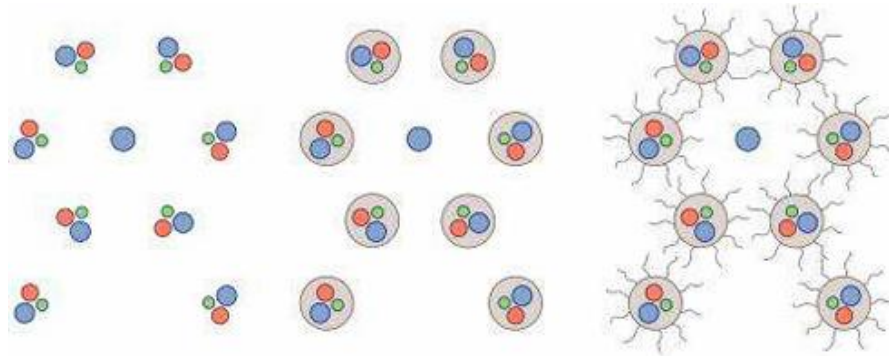
It assess

both **the degree of fibrosis**

**(ranging from no fibrosis to cirrhosis)**

and

**the histological activity score**

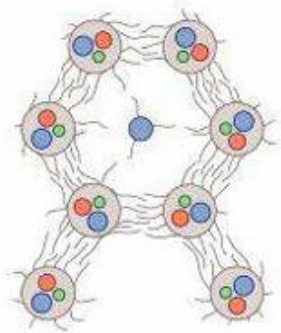**(reflecting inflammation and damage)**
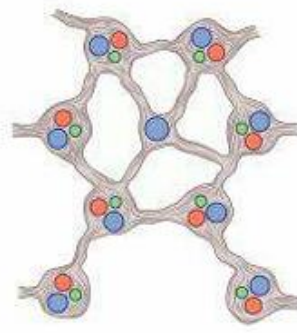
# Metavir scoring system



F0: No fibrosis

F1: Fibrous portal expansion

F2: Fibrous portal expansion with few bridges or septa

F3: Severe fibrosis with numerous bridges or septa

F4: Cirrhosis

Specific **serum fibrosis markers**
such as
the **Enhanced Liver Fibrosis panel**
may also be used.

Hyaluronic acid (HA), procollagen III N-terminal peptide (PIIINP), and tissue inhibitor of metalloproteinase-1 (TIMP-1)

## 2. Imaging:

**Ultrasound**

is most often used

and provides

a **qualitative assessment**

of **hepatic fat** content.

# Grading of Fatty Liver on Ultrasound:

- **Grade 0** (Absent):

  Normal liver echotexture, no noticeable increase in brightness.

- **Grade 1 (Mild):**

  Slightly increased echogenicity, with normal visualization of the diaphragm and portal vein walls.

# Grading of Fatty Liver on Ultrasound:

- **Grade 2 (Moderate):**
  Moderately increased echogenicity, with some blurring of the portal vein walls and impaired visualization of the diaphragm.

- **Grade 3 (Severe):**
  Significantly increased echogenicity, with poor visualization of the diaphragm and deep hepatic structures.

**Sensitivity** of
**ultrasound** is limited
when **fewer than**
**33%** of **hepatocytes**
are
**steatotic**.

**CT, MRI** or **MR spectroscopy**

offer **greater sensitivity**

for detecting **lesser degrees** of

**steatosis**

but these are

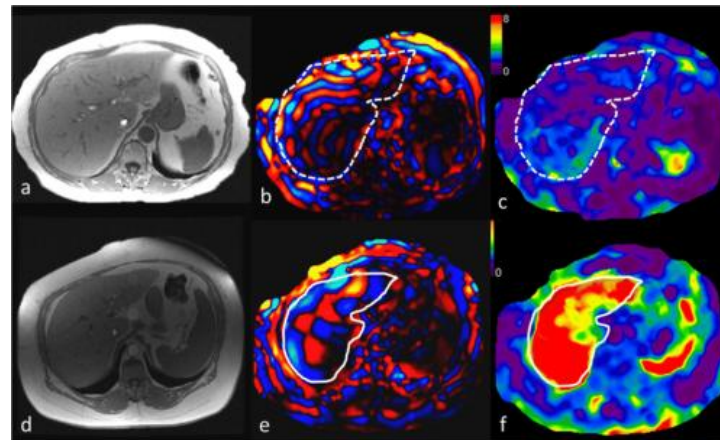**resource-intensive**

and

**not widely used**.

**No routine imaging** modality
can distinguish
**simple steatosis** from
**steatohepatitis**
or
accurately quantify
hepatic fibrosis short of
**cirrhosis**.

# 3. Transient elastography (TE):

## Transient elastography

also known as **FibroScan**

is often used

with **indeterminate fibrosis scores**.

The **fibroscan results**-

**Liver stiffness measurements (LSM)** are typically reported in **kilopascals (kPa).**

These are **categorized**
into **fibrosis scores**,
ranging from **F0 to F4**,
indicating **the severity of fibrosis.**

**Liver stiffness** can predict
the likelihood of
**advanced fibrosis**
and
is well validated in
**NAFLD**
although readings may get
less reliable with **BMI over 40**.

# Controlled Attenuation Parameter (CAP)

is an **ultrasound-based** technique which can be performed simultaneously with **TE** to quantify **steatosis** **non-invasively**.
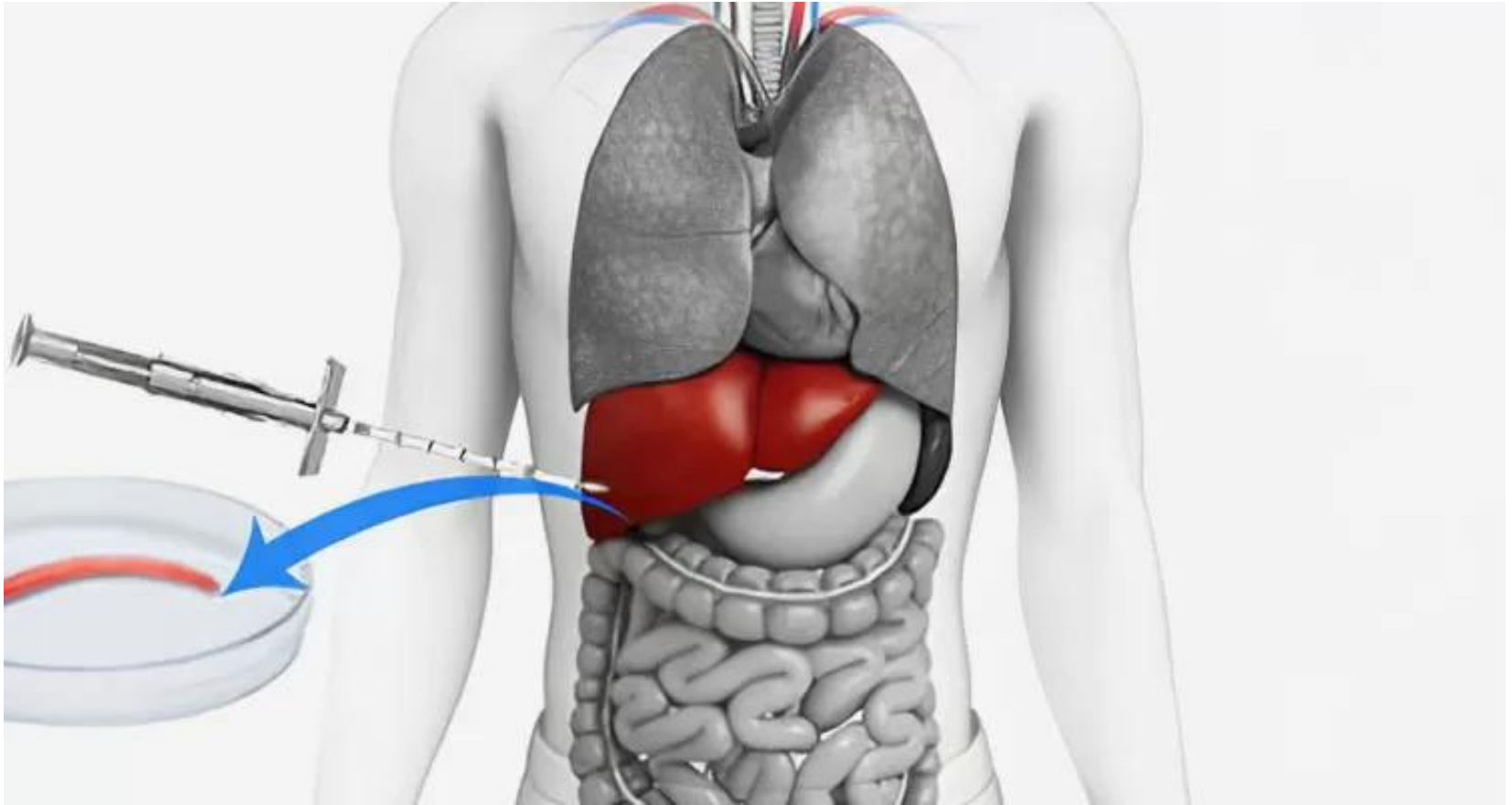
## 4. Liver biopsy:

**Liver biopsy** remains the
**'gold standard'**
investigation for
**diagnosis** and **assessment** of
degree of **inflammation**
and
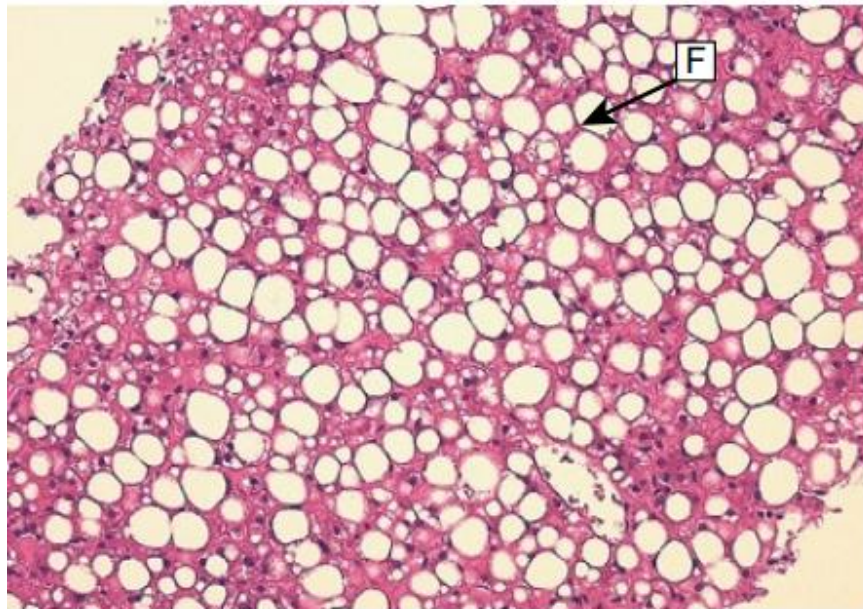**extent** of **liver fibrosis**.
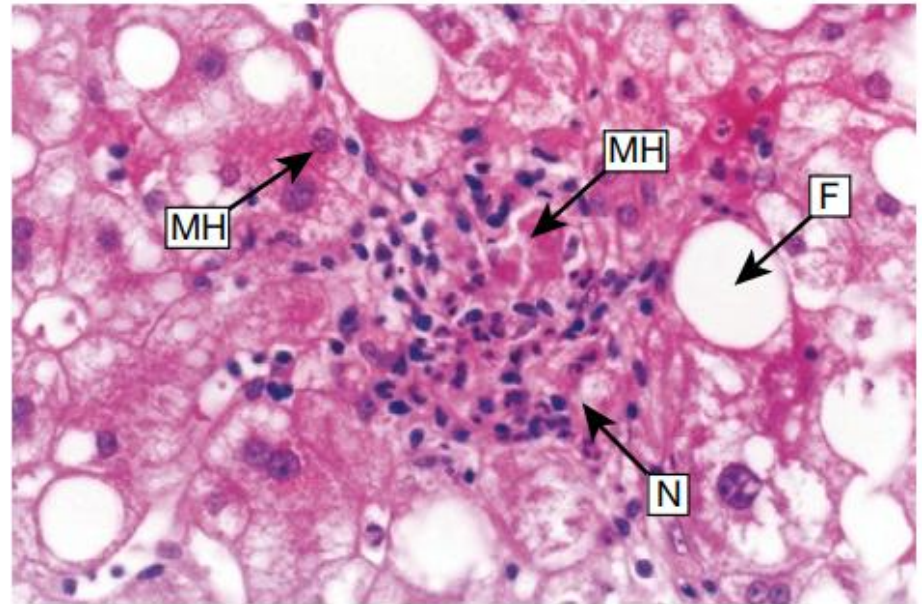
# Liver biopsy

# Liver biopsy

# Histology of non-alcoholic fatty liver disease



Large **fat droplets (F)** fill hepatocytes but there is **no inflammation**



**Fat is associated with an inflammatory infiltrate of neutrophils (N) and dense pink Mallory's hyaline (MH)**
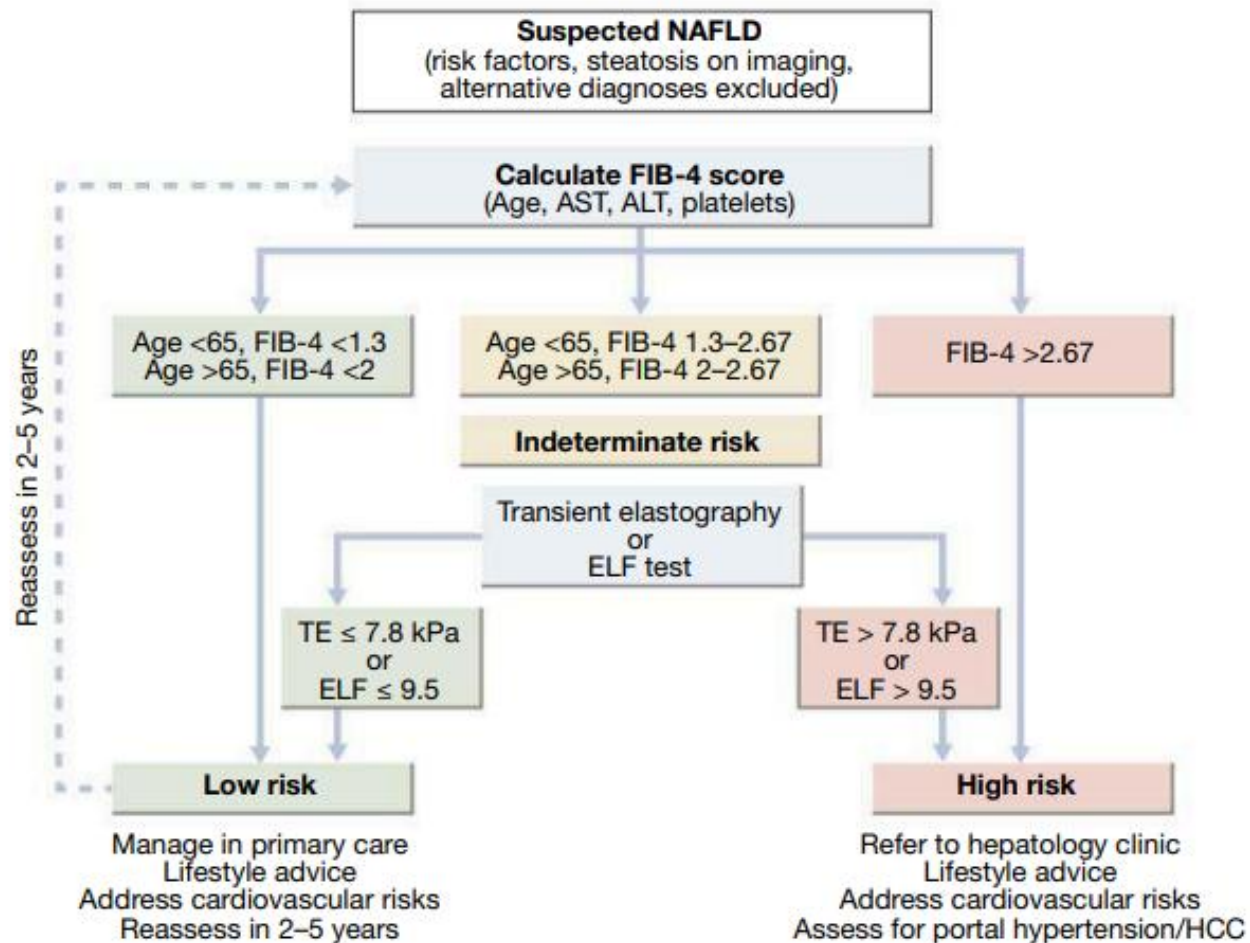
**Fig. 24.32 Assessment and risk stratification of patients with non-alcoholic fatty liver disease (NAFLD).** (ALT = alanine aminotransferase; AST = aspartate aminotransferase; ELF = enhanced liver fibrosis; FIB-4 = fibrosis-4; HCC = hepatocellular carcinoma; TE = transient elastography)

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# Management

**NAFLD**

is a **marker**

of the

**metabolic syndrome**.

Identification of **NAFLD** should **prompt screening** for and **treatment** of **cardiovascular risk factors** in all patients.

# 1. Non-pharmacological treatment

**Current treatment** comprises –

**Lifestyle interventions** to -
      promote **weight loss** and
      improve **insulin sensitivity** through
 a. **Dietery changes**
 b. **Physical exercise**.
    **(Cornerstone of management )**

**Sustained weight reduction and physical exercise**:

**Sustained weight reduction** of

**7–10%** is

associated with

**significant improvement** in

**histological**

and

**biochemical NASH severity**.

# 2. Pharmacological treatment

**No** **pharmacological agents**
are **currently licensed**
specially for
**NAFLD therapy**.

**Treatment** directed at
**coexisting**
**metabolic disorders**,
such as
**dyslipidaemia** and
**hypertension**
should be given.

Although

**HMG-CoA reductase inhibitors (statins)**

**do not ameliorate**

**NAFLD**,

may be used safely

to **treat**

**dyslipidaemia**.

Specific **insulin-sensitising agents**,

in particular **glitazones**

may help

**selected patients**.

Positive results with
**high-dose vitamin E** **(800U/day)**
have been tempered by
evidence that **high doses** may be
associated with an
**increased risk of prostate cancer**
and
all-cause **mortality**
which has **limited** its **use**.

**Obeticholic acid**

**(farnesoid X receptor agonist)**

appears to **improve**

**fibrosis**

in **non-cirrhotic NASH** in an

**interim analysis**

but

the trial is **ongoing**.

# *Take home message*

# *Take home message*

**Unhealthy lifestyle and habits –
risk of <span style="color:red">liver damage</span>**

# *Take home message*

## Sedentary Workers

A job is considered sedentary if

**walking** and **standing**

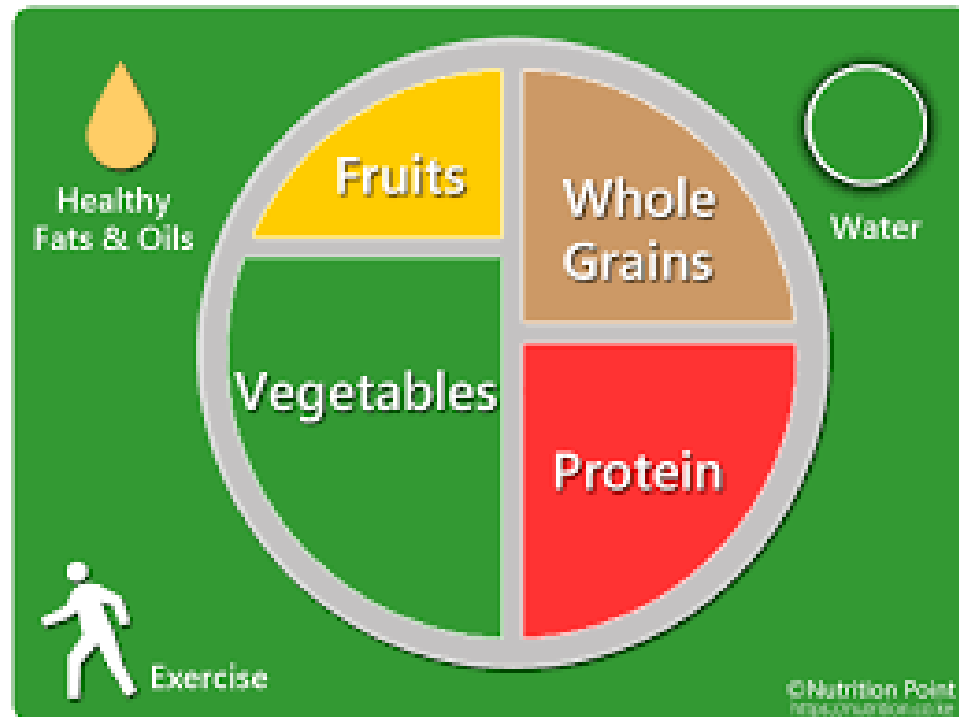only take up about

**two hours**

in an **eight hour** work day.

# *Take home message*

- Balanced diet

# *Take home message*

**NAFLD** is a growing **public health concern**.

- **Early detection** & **lifestyle changes** are crucial.

- **Public health awareness** & **multidisciplinary approach** is needed.