

Lipoprotein Subfractions

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Food and Drug Administration

Lipoprotein Subfractions

The identification of lipid fractions

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The identification of lipid fractions

The cholesterol pathway

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Effects of cholesterol

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Subfraction recommendations

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Subfraction recommendations

Conclusions

Literature Review

Literature Review

PUBNET and MedLine

Literature Review

PUBNET and MedLine

**Keywords – Lipoprotein, Lipoprotein Fractions,
Lipoprotein Subfractions, LDL, HDL,
Cholesterol and Electrophoresis**

Literature Review

PUBNET and MedLine

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Lipoprotein Subfractions, LDL, HDL,
Cholesterol

**Some articles selected were cited from other references
that were found on PubNet and MedLine**

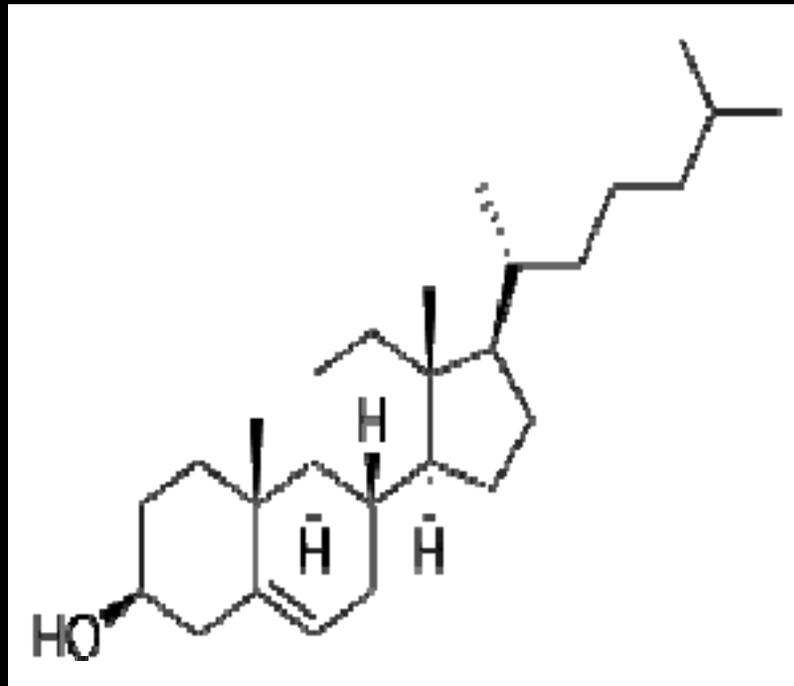
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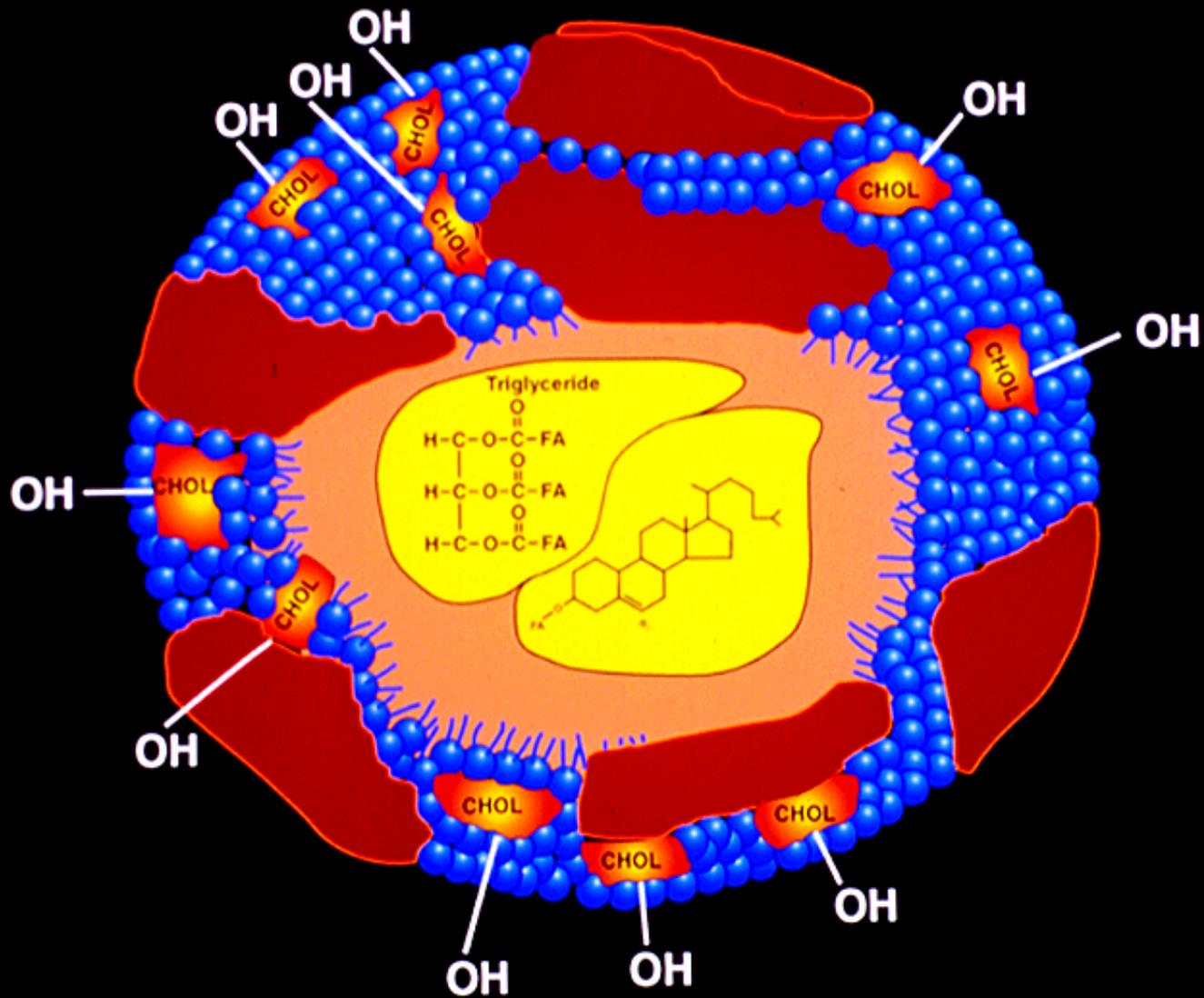
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**A complete list of references used is available in the
executive summary**

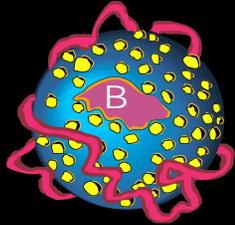


CHOLESTEROL

SCHEMATIC MODEL OF LIPOPROTEIN

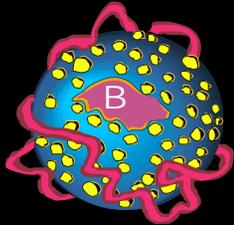


Types of Lipoproteins

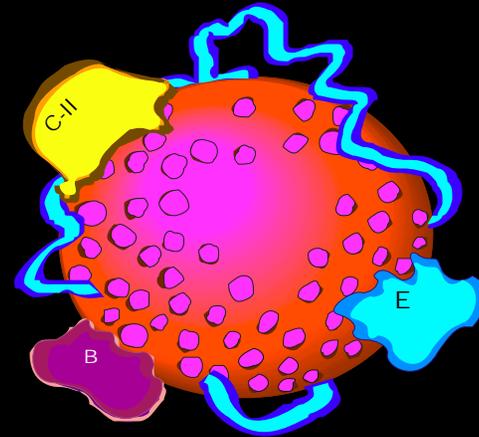


LDL

Types of Lipoproteins

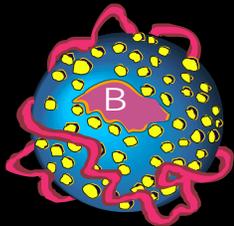


LDL

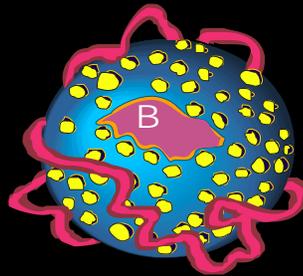


VLDL

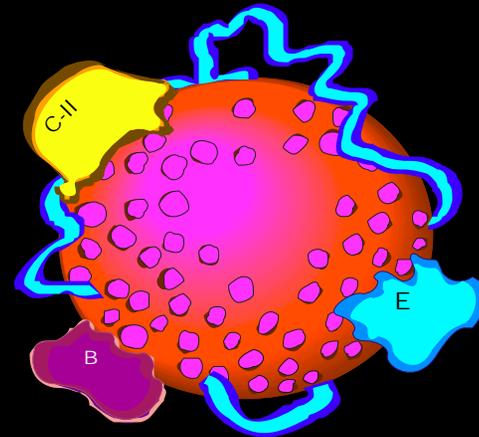
Types of Lipoproteins



LDL

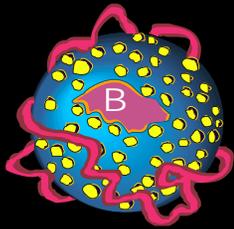


IDL

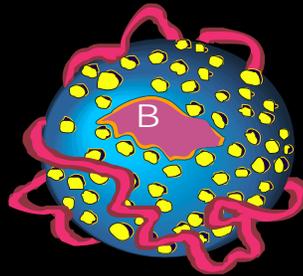


VLDL

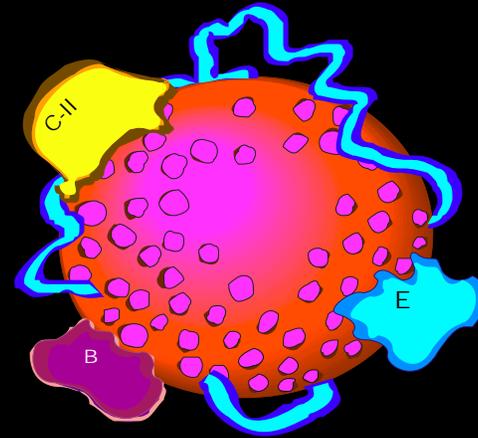
Types of Lipoproteins



LDL



IDL

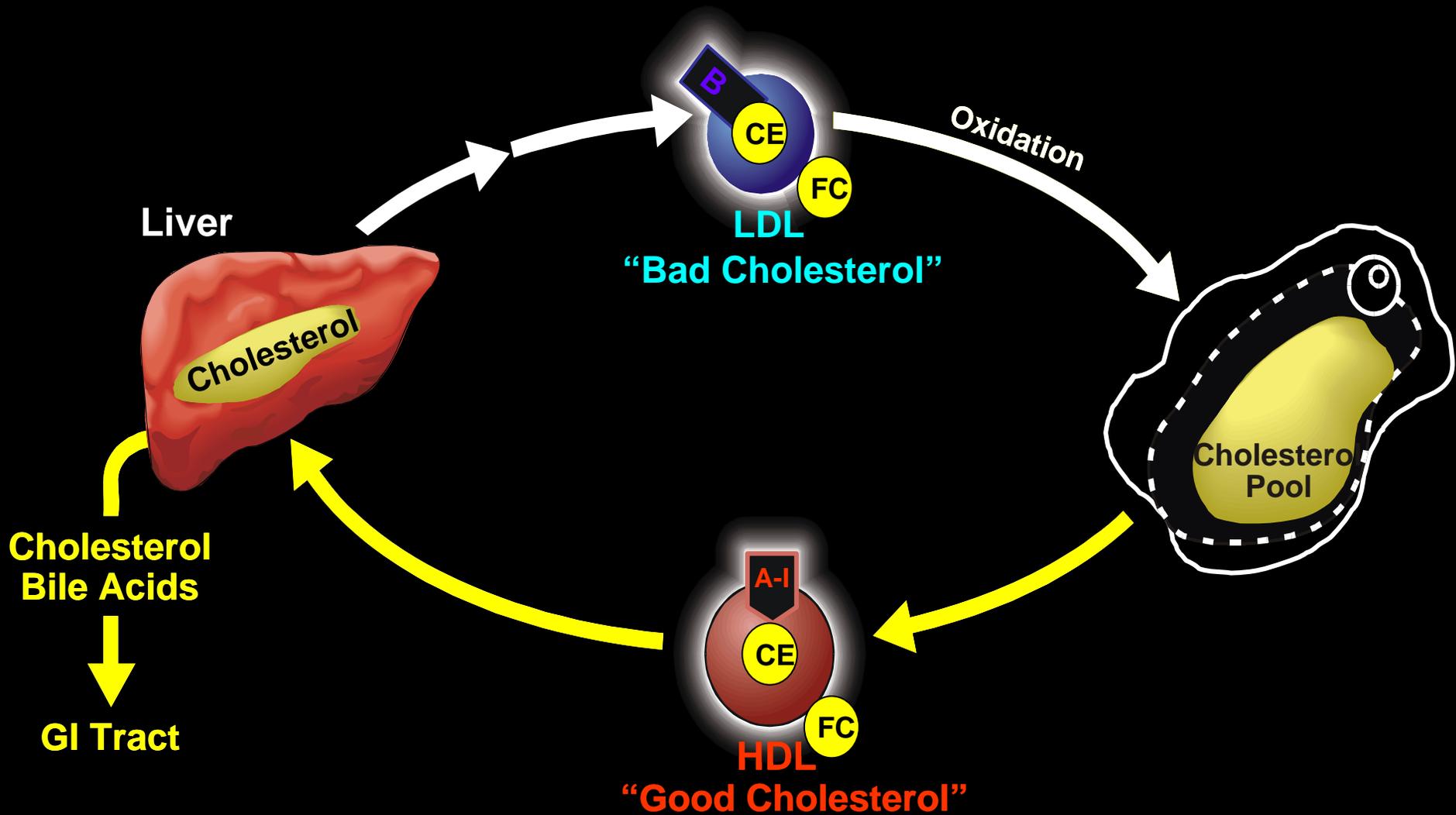


VLDL

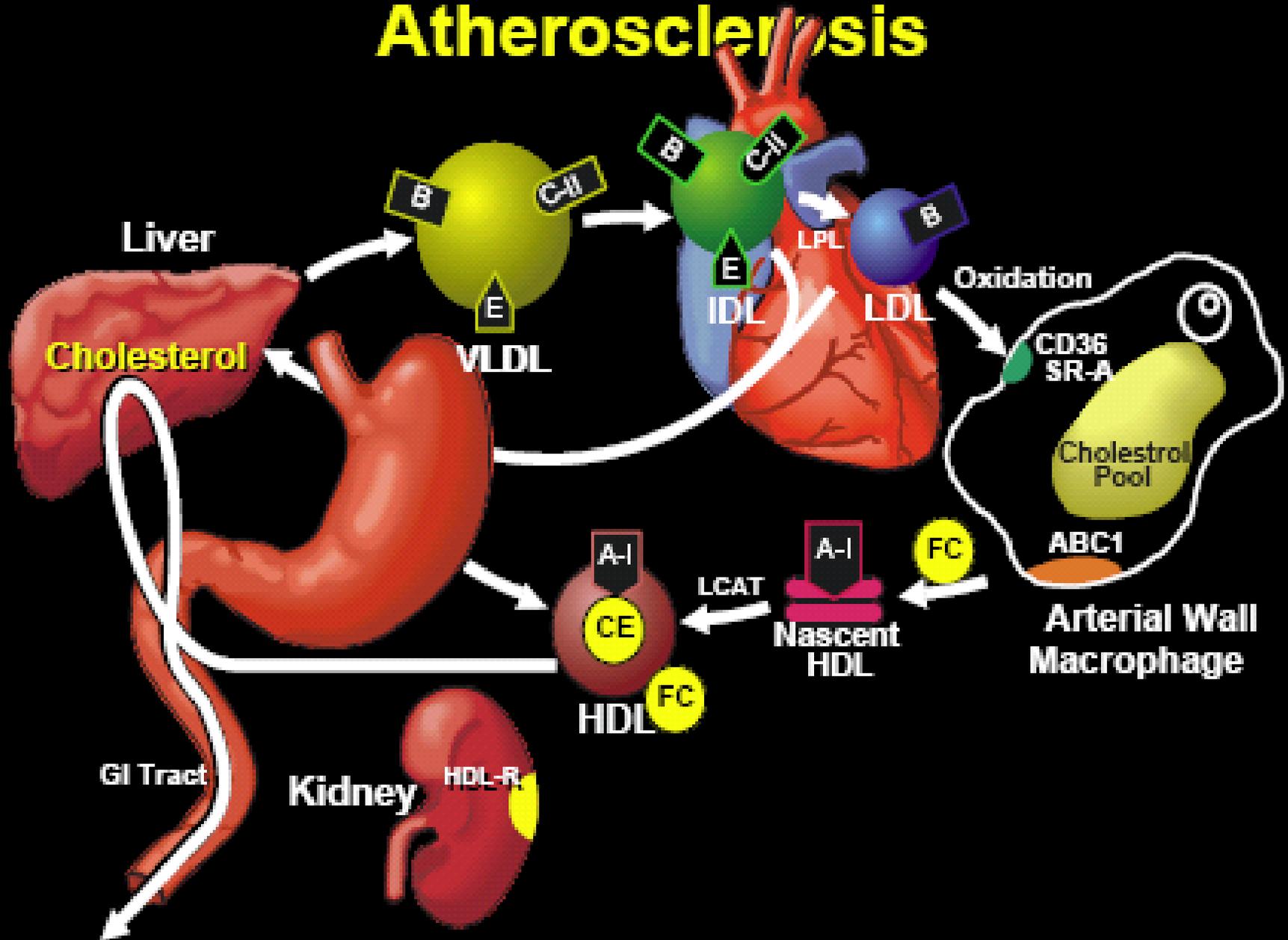


HDL

Cholesterol Metabolism

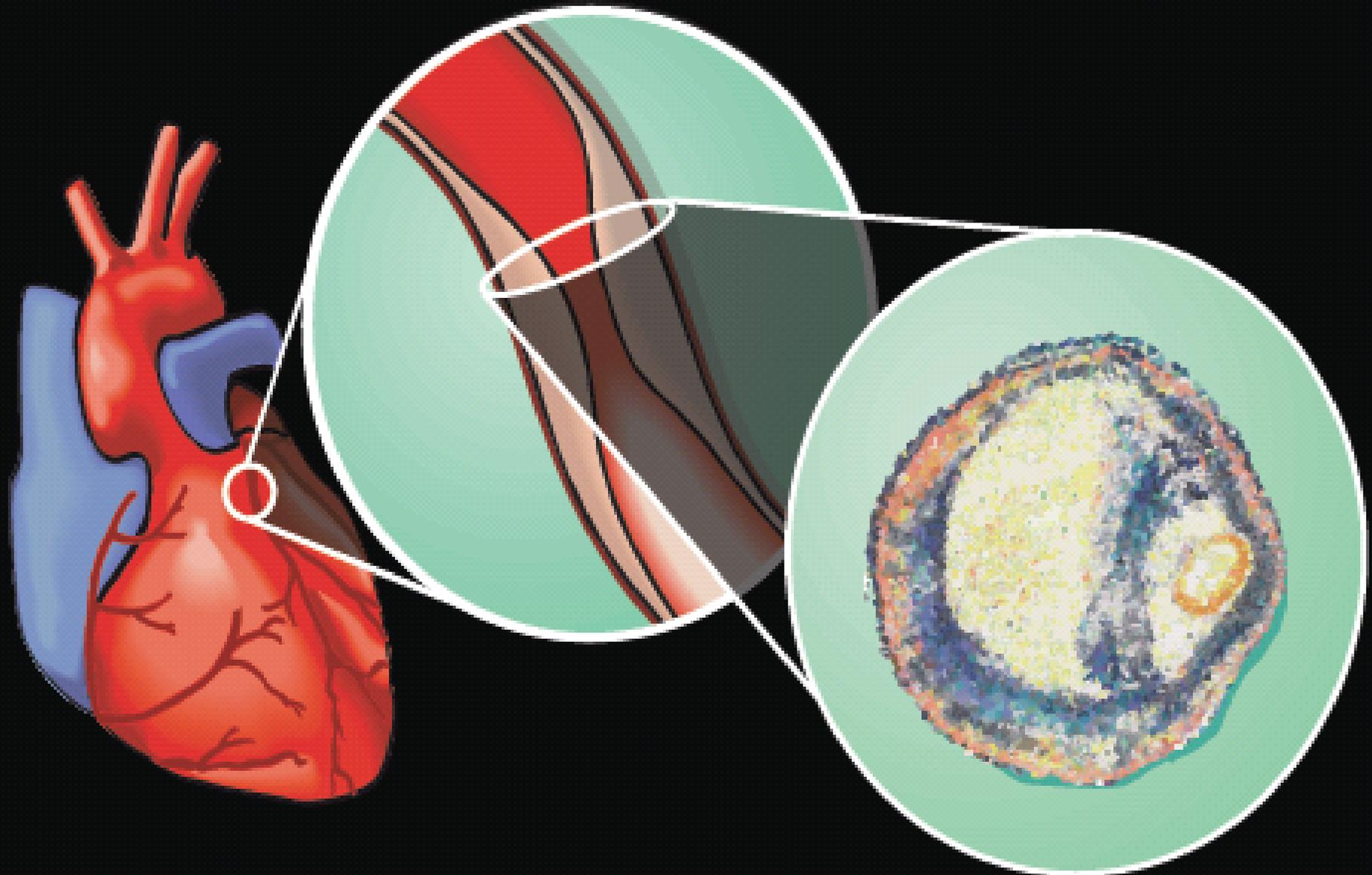


Lipid Metabolism and Atherosclerosis



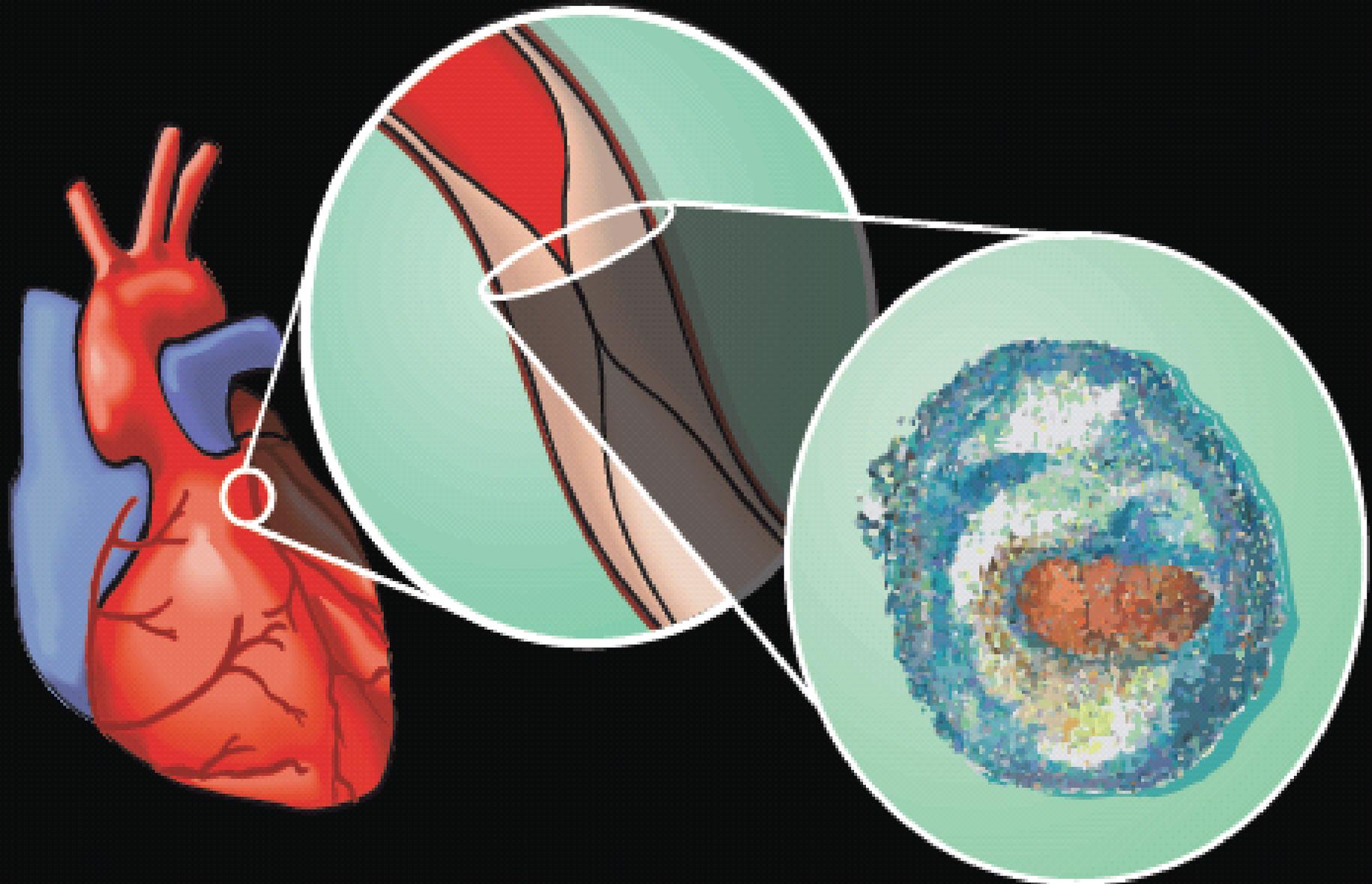
Cardiovascular Disease

Unstable Vulnerable Plaque



Cardiovascular Disease

Acute Heart Attack



For decades, cardiovascular disease has been a major cause of death and disability in developed countries –

WHO ,World Health Report 2002

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WHO, World Health Report 2002

Despite significant reduction in mortality due to cardiovascular disease in recent years, it remains the leading cause of death in the United States –

AHA, 2006 Heart and Stroke Statistics

Public Health Initiatives

**The American Heart Association
Prevention Conferences**

**The National Cholesterol Education
Program Adult Treatment Panel III**

**The National Academy of Clinical
Biochemistry (draft)**

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Global Risk Factors

Framingham Risk Score

Measurable Cardiac Risk Factors

Total Cholesterol

Low Density Lipoprotein Cholesterol (LDL-C)

High Density Lipoprotein Cholesterol (HDL-C)

Measurable Cardiac Risk Factors

Total Cholesterol

Low Density Lipoprotein Cholesterol (LDL-C)

High Density Lipoprotein Cholesterol (HDL-C)

Measurable Cardiac Risk Factors

Total Cholesterol

Low Density Lipoprotein Cholesterol (LDL-C)

High Density Lipoprotein Cholesterol (HDL-C)

Candidate Biomarkers

C – reactive protein

Factors V, VII, VIII

LDL Subfractions

Homocysteine

Apolipoprotein AI

Infectious Agents

D-Dimer

Lipoprotein (a)

HDL Subfractions

Microalbuminuria

Apolipoprotein B

Apolipoprotein E

Subtype

Candidate Biomarkers

LDL Subfractions

HDL Subfractions

Purpose

**The Purpose of this panel meeting
is to obtain input and
recommendations of the analytical
and clinical validity of lipid
subfraction diagnostics assays**

Lipid Profiles

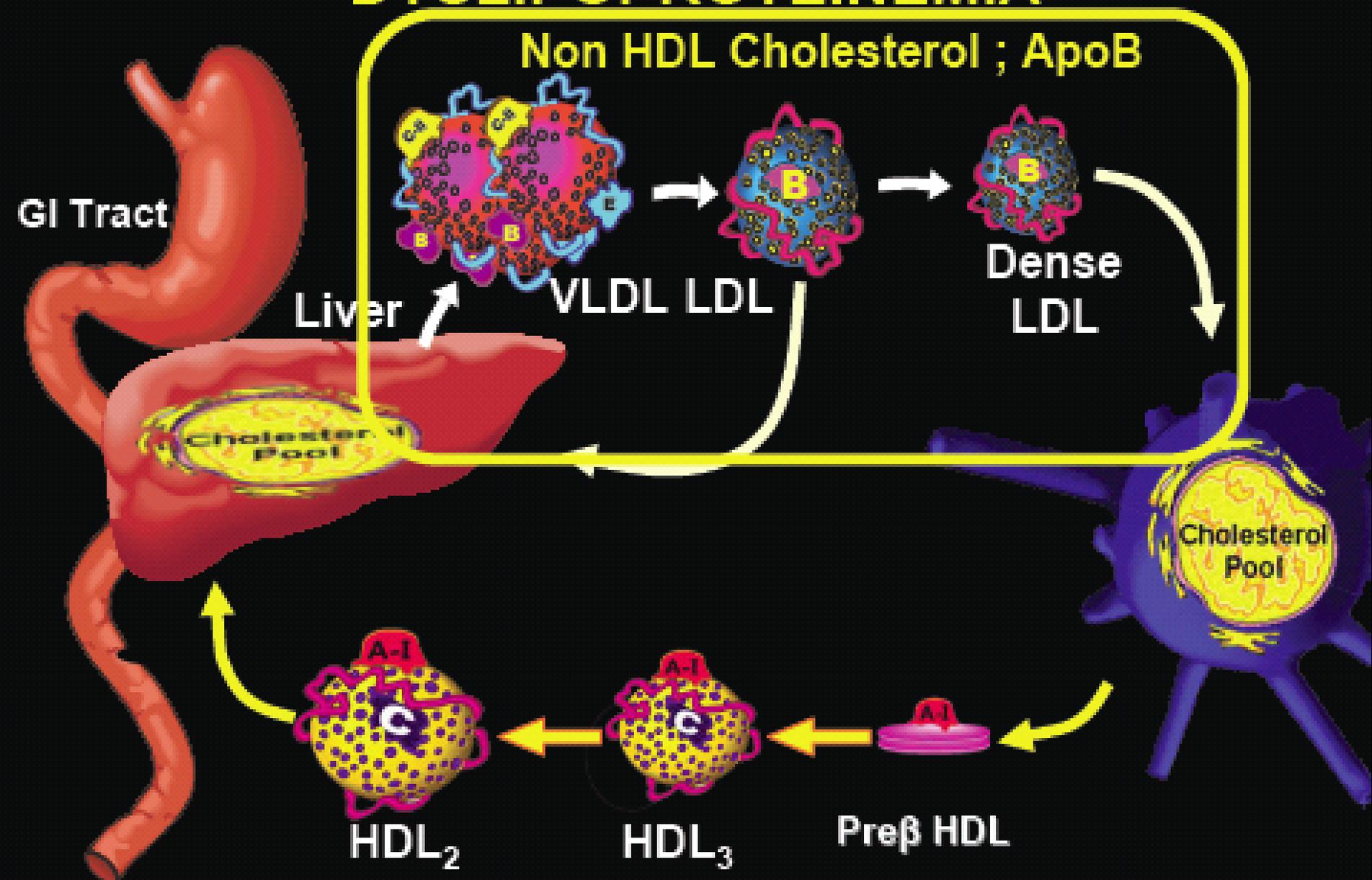
Profile/Pattern A

**Lower risk for
Cardiovascular
Disease**

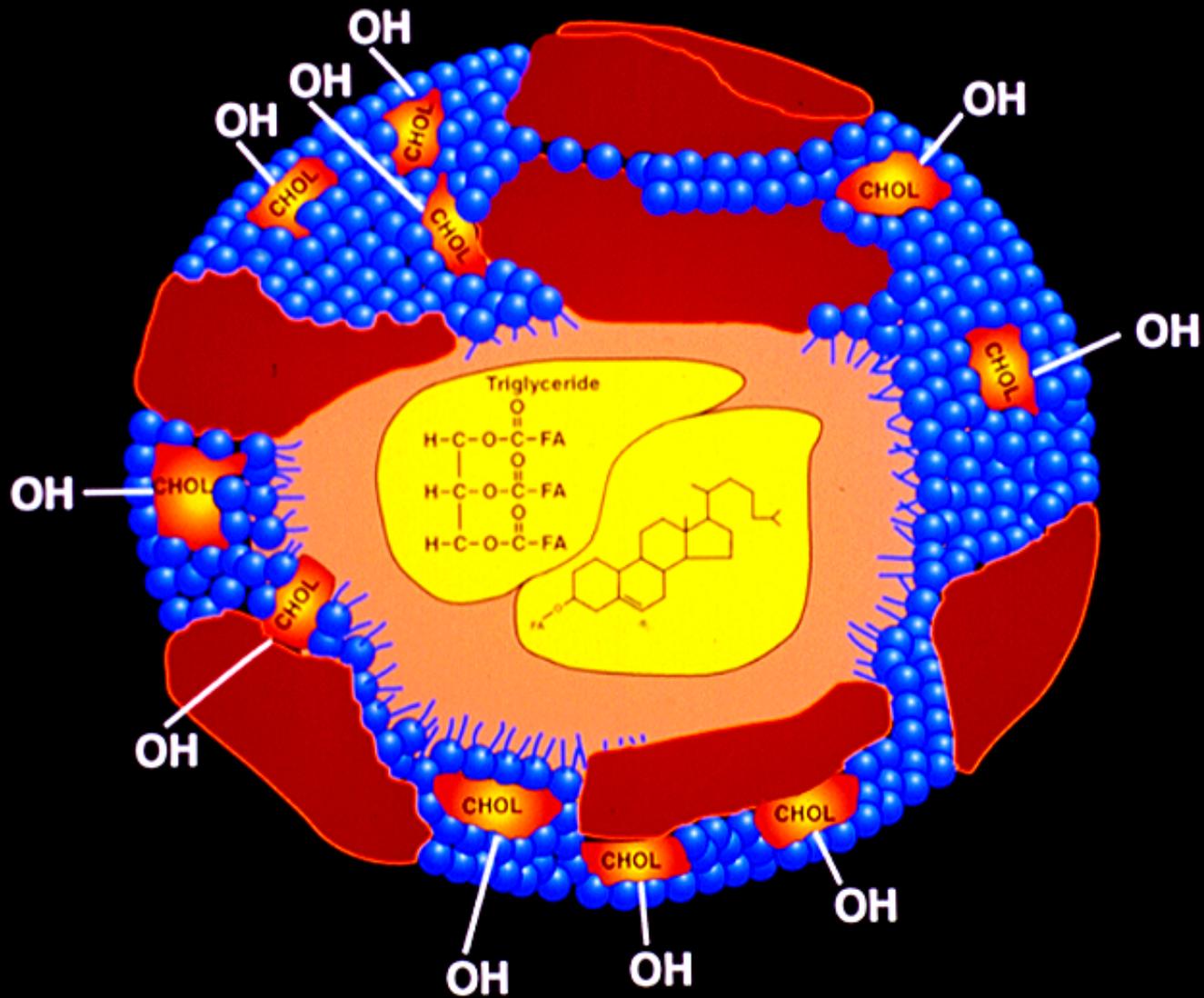
Profile/Pattern B

**Greater risk for
Cardiovascular
Disease**

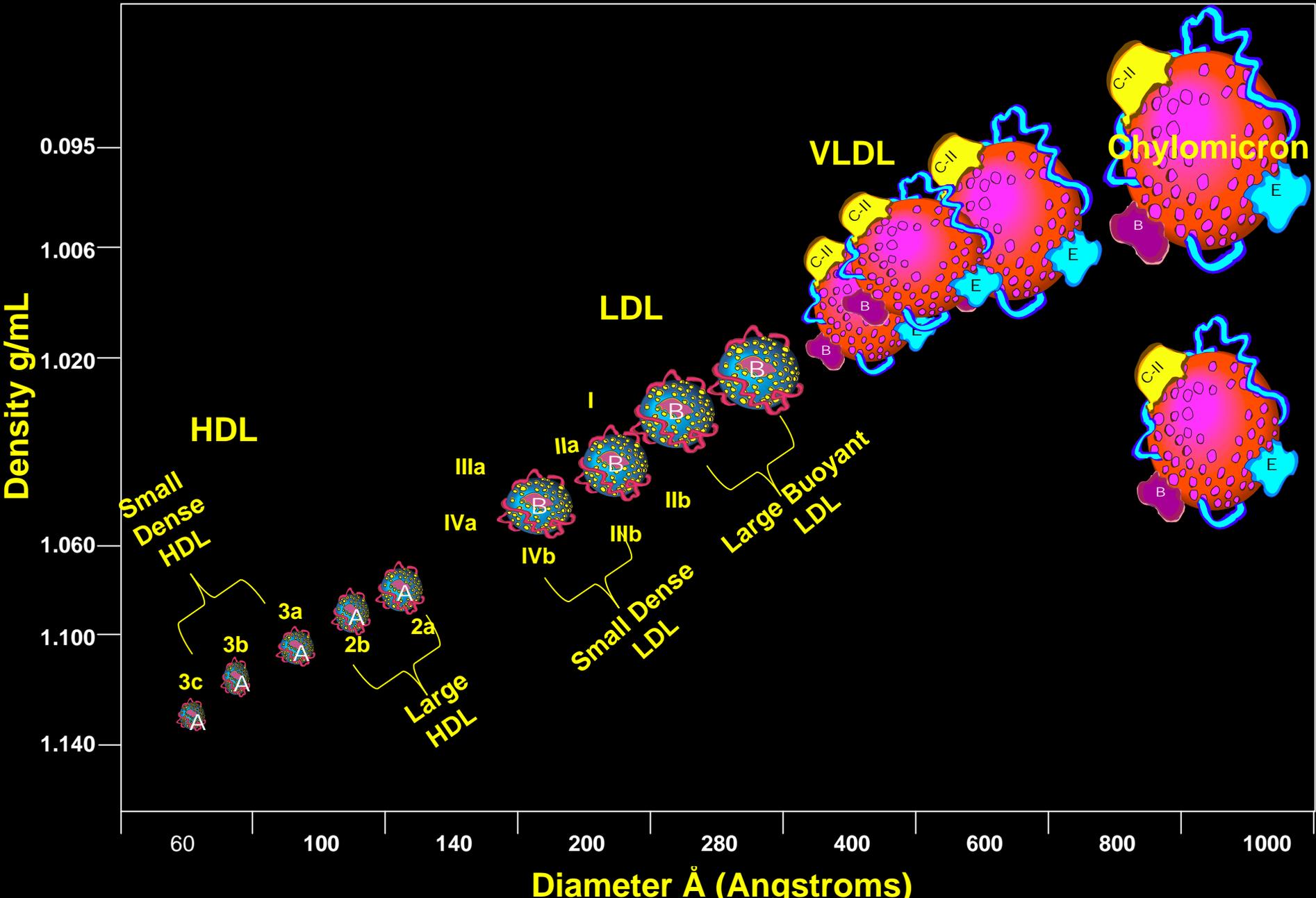
PLASMA LIPOPROTEINS IN ATHEROGENIC DYSLIPOPROTEINEMIA



SCHEMATIC MODEL OF LIPOPROTEIN



LIPOPROTEIN SUBFRACTIONS



LIPOPROTEIN SUBFRACTIONS

Some investigators have identified significant differences in interpretation of the different technologies used for lipid subfraction testing

Simplified Terminology of Lipoprotein Subclasses

Segmented Gradient Gel Electrophoresis

	Pattern B		Pattern Intermediate			Pattern A	
LDL Particles	IVb	IVa	IIIb	IIIa	IIb	IIa	I
HDL Particles	3c		3b	3a	2a	2b	

Nuclear Magnetic Resonance

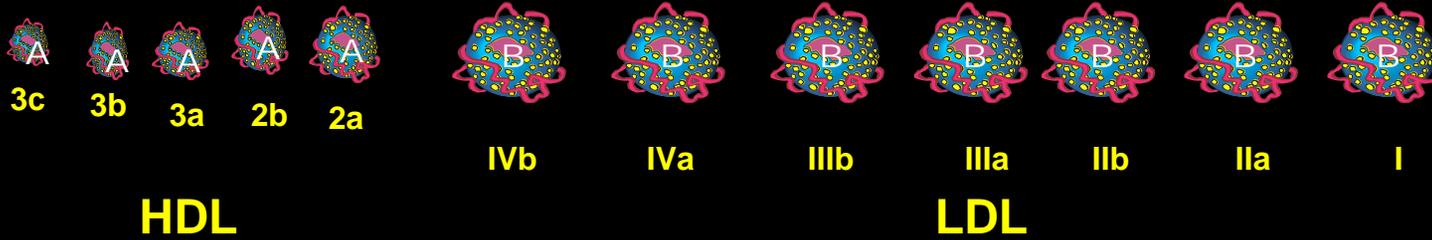
	Pattern B			Pattern A		
LDL Particles	L1		L2		L3	
HDL Particles	H1	H2	H3	H4	H5	
VLDL Particles	V1	V2	V3	V4	V5	V6

Short Single Vertical Automated with Gradient Ultracentrifugation

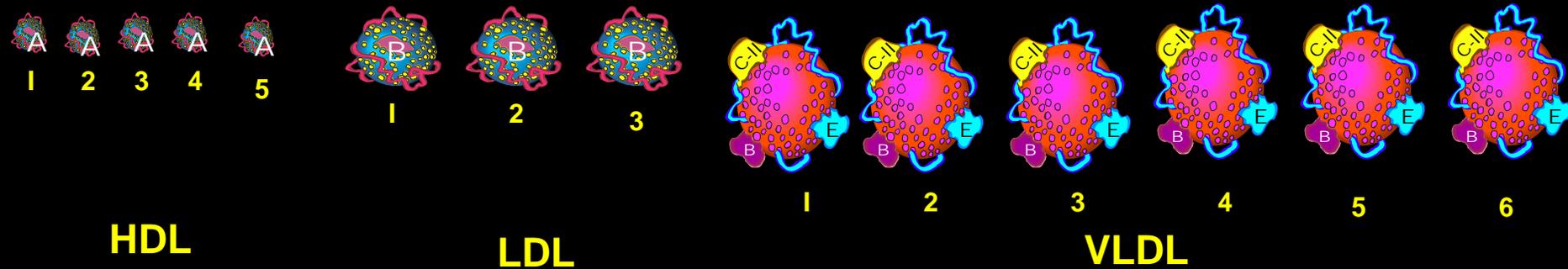
	Pattern B		Pattern A/B		Pattern A
LDL Particles	LDL4	LDL3	LDL2		LDL1
HDL Particles	HDL3(d,c,b,a)		HDL2 (a,b,c)		
VLDL Particles	VLDL 3b	VLDL 3a	VLDL 1 + 2		

LIPOPROTEIN SUBFRACTIONS

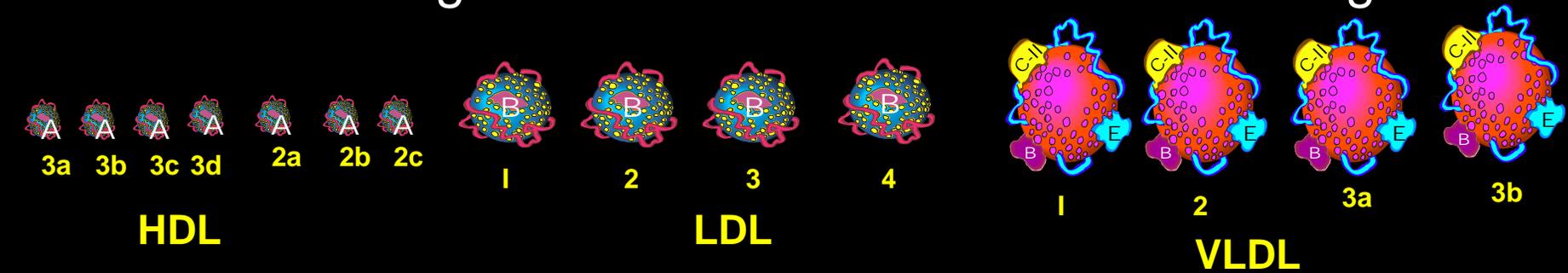
Segmented Gel Electrophoresis



Nuclear Magnetic Resonance



Short Single Vertical with Gradient Ultracentrifugation



Ensign et al Findings

Gradient Gel Electrophoresis (GGE)

Density Gradient Ultracentrifugation

Nuclear Magnetic Resonance

Tube Gel Electrophoresis

Ensign et al Findings

Gradient Gel Electrophoresis

Density Gradient Ultracentrifugation (DGU)

Nuclear Magnetic Resonance

Tube Gel Electrophoresis

Ensign et al Findings

Gradient Gel Electrophoresis

Density Gradient Ultracentrifugation

Nuclear Magnetic Resonance (NMR)

Tube Gel Electrophoresis

Ensign et al Findings

Gradient Gel Electrophoresis

Density Gradient Ultracentrifugation

Nuclear Magnetic Resonance

Tube Gel Electrophoresis (TGE)

Ensign et al Findings

Nomenclature

Expected Values

Number of Subfractions

Substantial Heterogeneity

Ensign et al Findings

Nomenclature

Expected Values

Number of Subfractions

Substantial Heterogeneity

Ensign et al Findings

Nomenclature

Expected Values

Number of Subfractions

Substantial Heterogeneity

Ensign et al Findings

Nomenclature

Expected Values

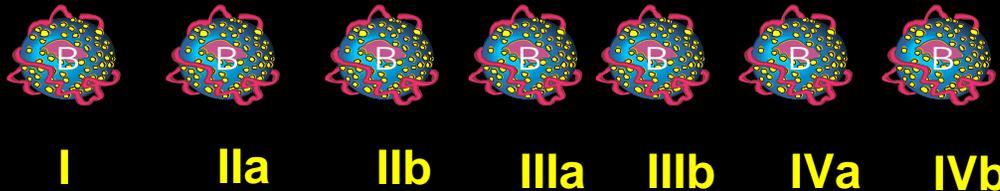
Number of Subfractions

Substantial Heterogeneity

Ensign et al Findings

Method 1 GGE

LDL is separated into 7 LDL subfractions based upon size and shape



Ensign et al Findings

Method 1 GGE

LDL is separated into 7 LDL subfractions based upon size and shape

LDL is separated into three patterns

26.35 – 28.5nm large LDL Pattern A

Ensign et al Findings

Method 1 GGE

LDL is separated into 7 LDL subfractions based upon size and shape

LDL is separated into three patterns

26.35 – 28.5nm large LDL Pattern A

25.75 – 26.34nm Intermediate LDL Pattern AB

Ensign et al Findings

Method 1 GGE

LDL is separated into 7 LDL subfractions based upon size and shape

LDL is separated into three patterns

26.35 – 28.5nm large LDL Pattern A

25.75 – 26.34nm Intermediate LDL Pattern AB

22.0 – 25.74 small LDL Pattern B

Ensign et al Findings

Method 1 GGE

LDL is separated into 7 LDL subfractions based upon size and shape

LDL is separated into three patterns

26.35 – 28.5nm large LDL Pattern A

25.75 – 26.34nm Intermediate LDL Pattern AB

22.0 – 25.74 small LDL Pattern B

LDL subfractions are reported as percentages based on the area under the curve for each subfraction

Ensign et al Findings

Method 1 GGE

With this method small LDL particles correspond to LDL IIIa and LDL IIIb

Ensign et al Findings

Method 1 GGE

With this method small LDL particles correspond to LDL IIIa and LDL IIIb

The findings of Ensign et al suggest that these subfractions, based upon GGE are indicators of the severity of the atherogenic profile

Ensign et al Findings

Method 2 DGU

Generates a series of absorbance curves
which produces 6 LDL subclasses



LDL 1



LDL 2



LDL 3



LDL 4



LDL 5



LDL 6

Ensign et al Findings

Method 2 DGU

Generates a series of absorbance curves which produces 6 LDL subclasses

Subclasses are identified as Class I (most buoyant) through Class 6 (most dense)

Ensign et al Findings

Method 2 DGU

Generates a series of absorbance curves which produces 6 LDL subclasses

Subclasses are identified as Class I (most buoyant) through Class 6 (most dense)

**LDL 1 and LDL2 comprise pattern type A and
LDL3 and LDL4 comprise pattern type B**

Ensign et al Findings

Method 3 NMR

Generates three LDL subclasses



L1



L2



L3

Ensign et al Findings

Method 3 NMR

Generates three LDL subclasses

No references are provided for the basis of risk categories

Ensign et al Findings

Method 4 TGE

LDL scan contains 7 possible LDL subfractions



LDL 1



LDL 2



LDL 3



LDL 4



LDL 5



LDL 6



LDL 7

Ensign et al Findings

Method 4 TGE

LDL scan contains 7 possible LDL subfractions

Lipoproteins are separated to yield a score

Ensign et al Findings

Method 4 TGE

LDL scan contains 7 possible LDL subfractions

Lipoproteins are separated to yield a score

**Specific ranges of scores correspond to
different LDL Patterns**

Ensign et al Findings

Method 4 TGE

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**Specific ranges of scores correspond to
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Normal <5.5 (Pattern A)

Ensign et al Findings

Method 4 TGE

LDL scan contains 7 possible LDL subfractions

Lipoproteins are separated to yield a score

**Specific ranges of scores correspond to
different LDL Patterns**

Normal <5.5 (Pattern A)

Intermediate 5.5 – 8.5 (Pattern AB)

Ensign et al Findings

Method 4 TGE

LDL scan contains 7 possible LDL subfractions

Lipoproteins are separated to yield a score

**Specific ranges of scores correspond to
different LDL Patterns**

Normal <5.5 (Pattern A)

Intermediate 5.5 – 8.5 (Pattern AB)

Atherogenic >8.5 (Pattern B)

Ensign et al Findings

Method 4 TGE

Does not measure LDL particle size directly but estimates size by comparing electrophoretic mobility to the mobility of particles of known sizes

Ensign et al Findings

Nomenclature

Expected Values

Number of Subfractions

Substantial Heterogeneity

Ensign et al Findings

Gradient Gel Electrophoresis



I IIa IIb IIIa IIIb IVa IVb

Density Gradient Ultracentrifugation



LDL 1 LDL 2 LDL 3 LDL 4 LDL 5 LDL 6

Nuclear Magnetic Resonance



L1 L2 L3

Tube Gel Electrophoresis

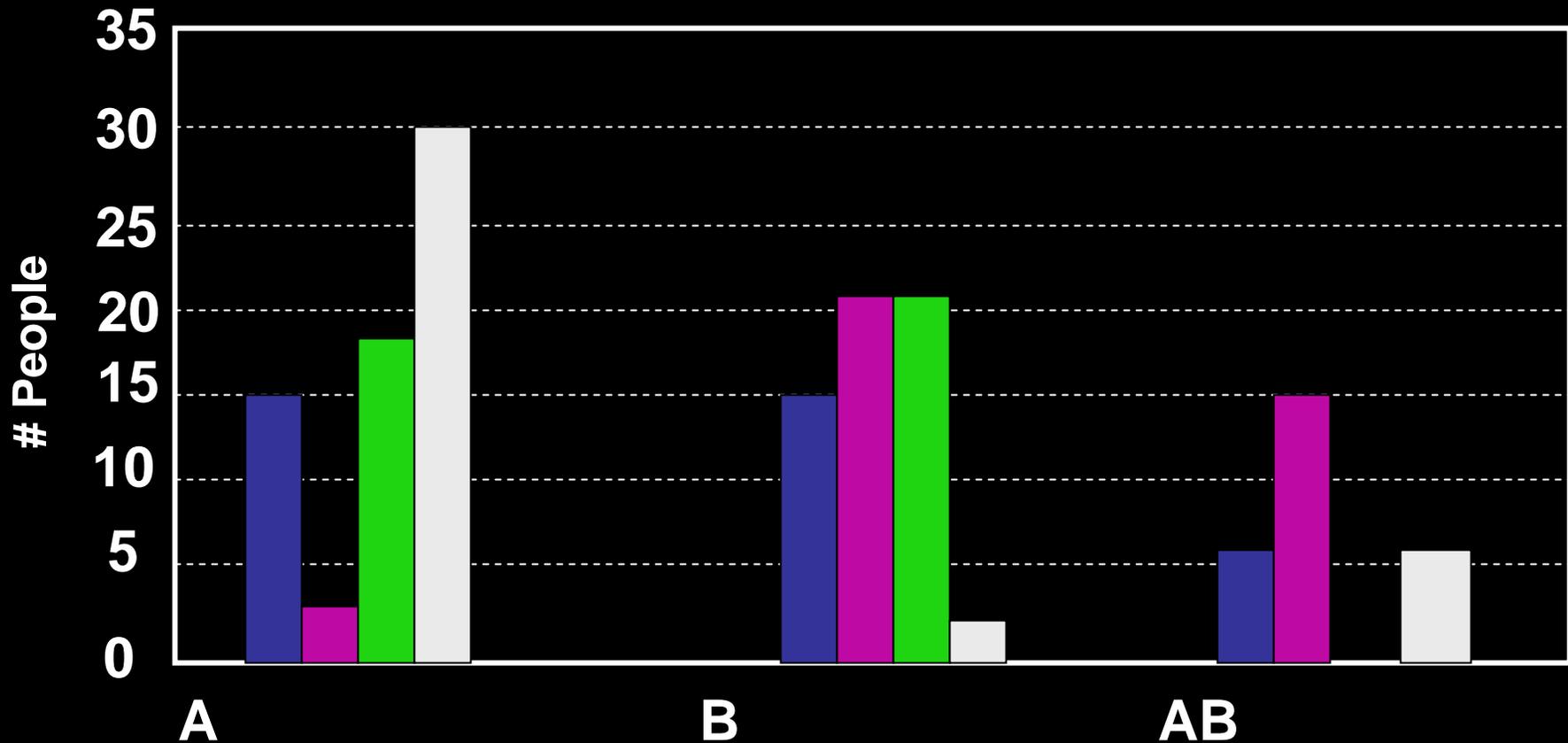


LDL 1 LDL 2 LDL 3 LDL 4 LDL 5 LDL 6 LDL 7

Ensign et al Findings

Distribution of LDL Phenotypes

GGE DGU NMR TGE



Ensign et al Conclusions

Nomenclature

Expected Values

**Total number of subfractions identified is
method dependent**

**A substantial heterogeneity of interpretations
with complete agreement in only 8% of
samples**

NCEP ATP III Guidelines

Established a link between LDL levels and CVD

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Identify the combination of elevated triglycerides and Low HDL-C as an associated risk of CVD

NCEP ATP III Guidelines

Established a link between LDL levels and CVD

Identify the combination of elevated triglycerides and Low HDL-C as an associated risk of CVD

Recommend treatment of individuals at high risk based upon LDL-C and triglyceride values

Other Study Findings

**As LDL increases small dense LDL
subfractions increase**

Cromwell WC, Otvos JD. Current Atherosclerosis Reports 2004

Campos H, et al. J Clin Endo Metab 1988;67:30-35.

Asztalos BF, et al. Biochimica et Biophysica Acta 1993

Asztalos BF, et al. Arterioscler Thromb Vasc Biol 2000

Asztalos BF, et al. Arterioscler Thromb Vasc Biol 2003

Asztalos BF, et al. Journal of Lipid Research. 2004

Asztalos BF, et al. Arterioscler Thromb Vasc Biol 2004

Asztalos BF, et al. Arterioscler Thromb Vasc Biol 2005

Otvos JD, et al. Am J Cardiol 2002.

Otvos JD, et al. Circulation 2006

Otvos JD. et al. Clin Lab 2002

Other Study Findings

As LDL increases small dense LDL subfractions increase

As HDL decreases there is a marked decrease in larger HDL particles

Other Study Findings

As LDL increases small dense LDL subfractions increase

As HDL decreases there is a marked decrease in larger HDL particles

Based upon these findings, it has been suggested that elevated LDL, elevated small dense LDL subfractions, low HDL and low HDL subfractions are predictive of CVD

LIPOPROTEIN SUBFRACTIONS

Although there is evidence that lipid subfraction profiles differ between individuals with established CVD and normolipidemic individuals, it is unclear to the FDA whether meaningful and reproducible diagnostic cutoffs for particle size, density and/or number can be established

Other Study Findings

Some investigators have observed that lipid subfraction reference ranges for patient risk for CVD (as defined by NCEP) vs normolipidemic patients have considerable overlap

HDL Fractions comparison

Normolipidemic

	HDL Large (mg/dL)	HDL Intermediate (mg/dL)	HDL Small (mg/dL)	HDL total (mg/dL)	Cholesterol total (mg/dL)
Range	8-43	18 – 44	0 - 12	40 - 89	110 - 199
Mean	21.7	30.4	4.3	56.5	166.2
SD	8.05	5.06	2.56	10.87	19.37
96% range	10 - 41.9	22.0 - 41.9	1.0 - 11.0	41.0 - 79.9	118.5 - 197.8

Dyslipidemic

	HDL Large (mg/dL)	HDL Intermediate (mg/dL)	HDL Small (mg/dL)	HDL total (mg/dL)	Cholesterol total (mg/dL)
Range	2 - 90	13 – 53	1 - 19	21 - 122	94 - 322
Mean	14.5	28.1	6.2	49.0	213.9
SD	10.3	7.01	3.05	15.54	39.17
96% range	3.8 - 37.0	16.8 - 43.2	1.0 - 12.6	27.0 - 85.8	124.6 - 299.4

NCEP Guidelines

The NCEP ATPIII guidelines recognize that small LDL particles have been identified as a component of atherogenic dyslipidemia, and that some studies have suggested that some HDL subfractions may make important contributions to CVD risk assessment.

NCEP Guidelines

The guidelines state that LDL particles are formed in large part as a response to elevated triglyceride. However, while these guidelines assert that LDL subfractions plus elevated triglyceride is associated with CVD, they also note that the ability of LDL subfractions to predict CVD independently of other risk factors is not well defined.

NCEP Guidelines

The guideline also points out that the clinical performance of HDL subfractions has not been established. As a result of this and the ready availability of standard methodologies, the ATPIII does not recommend the measurement of small lipid particles in routine practice.

NACB Draft Recommendations

In addition, the NACB recently proposed new guidelines for the use of several biomarkers for the assessment of CVD risk. The NACB is proposing the following three recommendations concerning lipid subclasses:

NACB Draft Recommendations

- 1. Lipid subclasses, especially the number or concentration of small dense LDL particles have been shown to be related to the development of initial coronary heart disease events, but the data analysis of existing studies are generally not adequate to show added benefit over standard risk assessment.**

NACB Draft Recommendations

[Classification/Weight of Evidence: The committee found that there is evidence and/or general agreement that measurement of lipid subclasses is not useful (and in some cases might be harmful) based on data obtained from multiple randomized clinical trials that involved large numbers of patients.]

NACB Draft Recommendations

- 2. There is insufficient data that measurement of lipid subclasses over time is useful to evaluate the effects of treatments.**

NACB Draft Recommendations

[Classification/Weight of Evidence: The committee found that there is conflicting evidence and/or divergence of opinion about the usefulness/efficacy of these assays, with the usefulness/efficacy of the test being less well established. This conclusion was based on a consensus of opinion of the experts in the field.]

NACB Draft Recommendations

- 3. Several methods are available to assess lipoprotein subclasses. Standardization is needed for this technology.**

NACB Draft Recommendations

[Classification/Weight of Evidence: The committee found that there is conflicting evidence and/or divergence of opinion about the usefulness/efficacy of standardization, with the weight of evidence/opinion being in favor of standardization. This conclusion was based on a consensus of opinion of the experts in the field.]

Conclusion

The proposed recommendations cited above and the published reports provide insight regarding the current understanding of the clinical usefulness of these types of assays and the strengths and weaknesses of these potential biomarkers.

FDA Comments

However, FDA's task when evaluating whether a novel assay should be cleared (or approved) is to determine whether the assay can be found substantially equivalent to existing assays (or is reasonably safe and effective for its intended use).

For that purpose we focus on the analytical and clinical validity of the assay based on the specific claim(s) that are made when promoting and labeling the device.

FDA Comments

The FDA seeks the advice of this panel regarding whether the clinical use of these devices pose a risk to the public health.

FDA also requests that the panel discuss the effectiveness of these devices to measure and diagnose lipid disorders and atherosclerosis.