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Small dense LDL and dyslipidemia

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Interreg
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Roles of lipoproteins in human body

Knowledge about:

- metabolism of lipoproteins (Lp)
- lipoprotein families (relation to Classification of Lp)
- biological role of Lp in human body (which is multiple) and includes:

  - ✓ **Formation of cellular and subcellular structures, Sources of energy,**
  - ✓ **Synthesis of steroid hormones,**
  - ✓ **Vitamin D and bile acids,**
  - ✓ **Immuno-modulatory effects of Lp,**
  - ✓ **Electro-mechanical characteristics,**
  - ✓ **Transport of vitamins,**
  - ✓ **Role of Lp in starvation,**
  - ✓ **Cellular apoptosis,**
  - ✓ **Aging**

Role of lipoproteins in human body

Knowledge about:

- pathomechanisms of generation of metabolic diseases
- interaction between Lp <–> vascular endothelium
- how Lp participate in creation and development of degenerative vascular diseases - their share in the atherogenic process
- atheroprotective characteristics of lipoproteins

Lipoproteins

- are the **transport system of lipids** in human body

Classes of Lp:

- **Chylomicrons**
- **VLDL** (very low density lipoproteins)
- **IDL** (intermediate density lipoproteins)
- **LDL** (low density lipoproteins)
- **Lp(a)** (lipoprotein (a))
- **HDL** (high density lipoproteins)

Risk factors of CVD development

The high level of cholesterol & cholesterol transporting Lp (LDL) are responsible for the development of CVD

- **Coronary heart disease - Myocardial infarction**
- **Stroke**
- **Peripheral artery diseases**

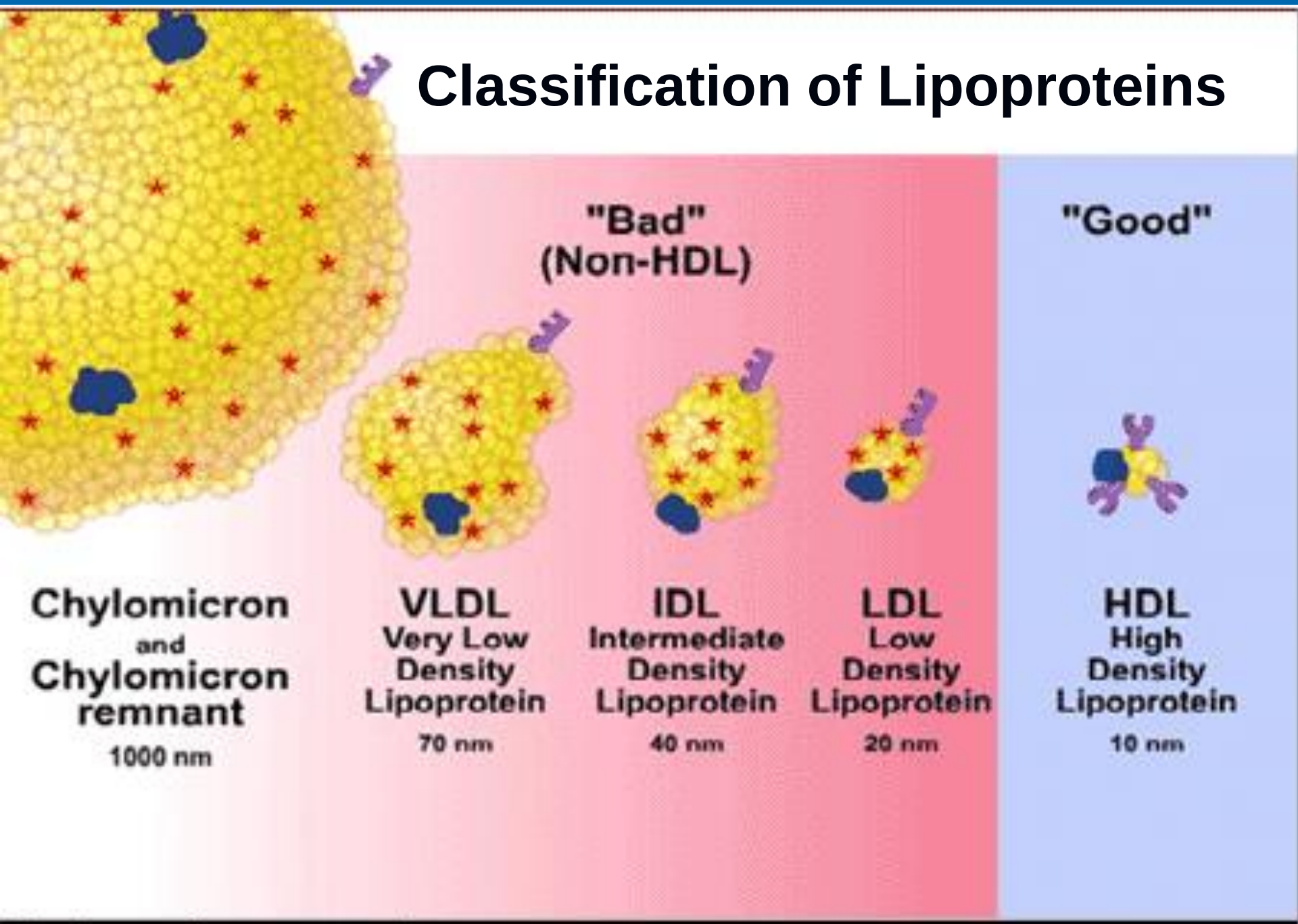
Risk factors of CVD development

Triglyceride-rich Lp and TG are considered to be atherogenic too:

- (Chylomicrons)
- **VLDL**
- **IDL**

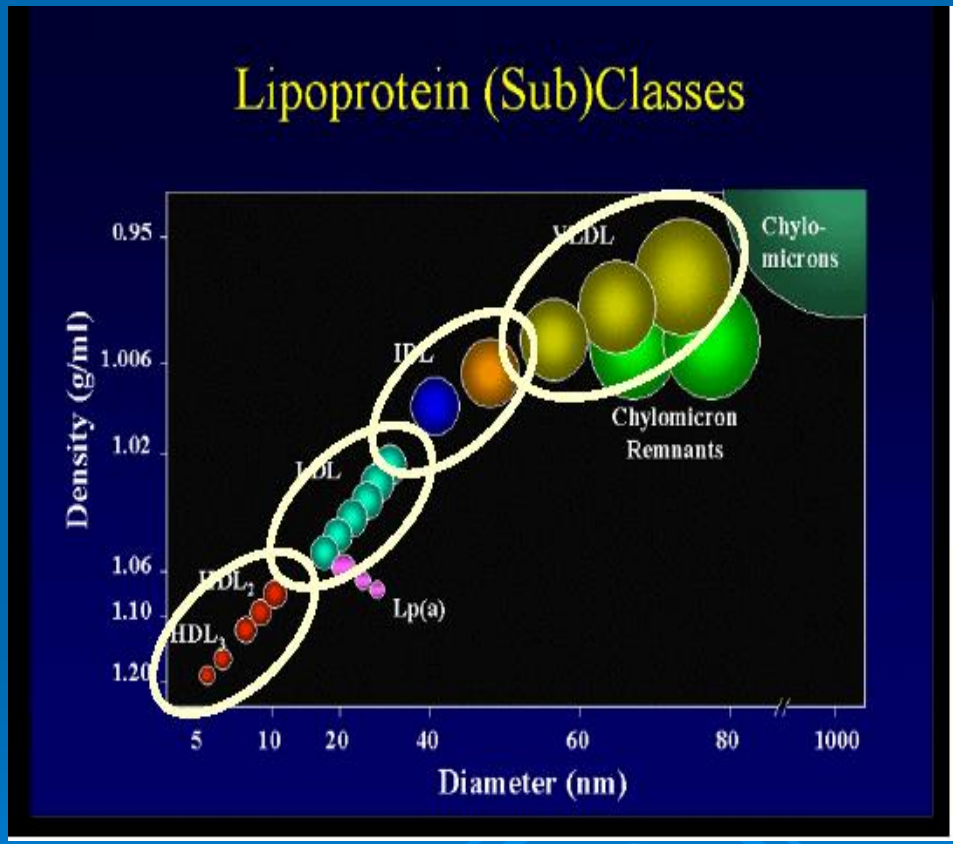
HDL – high density lipoproteins
- protective role against the generation and development of degenerative vascular damage

Classification of Lipoproteins



Heterogeneity of lipoprotein families

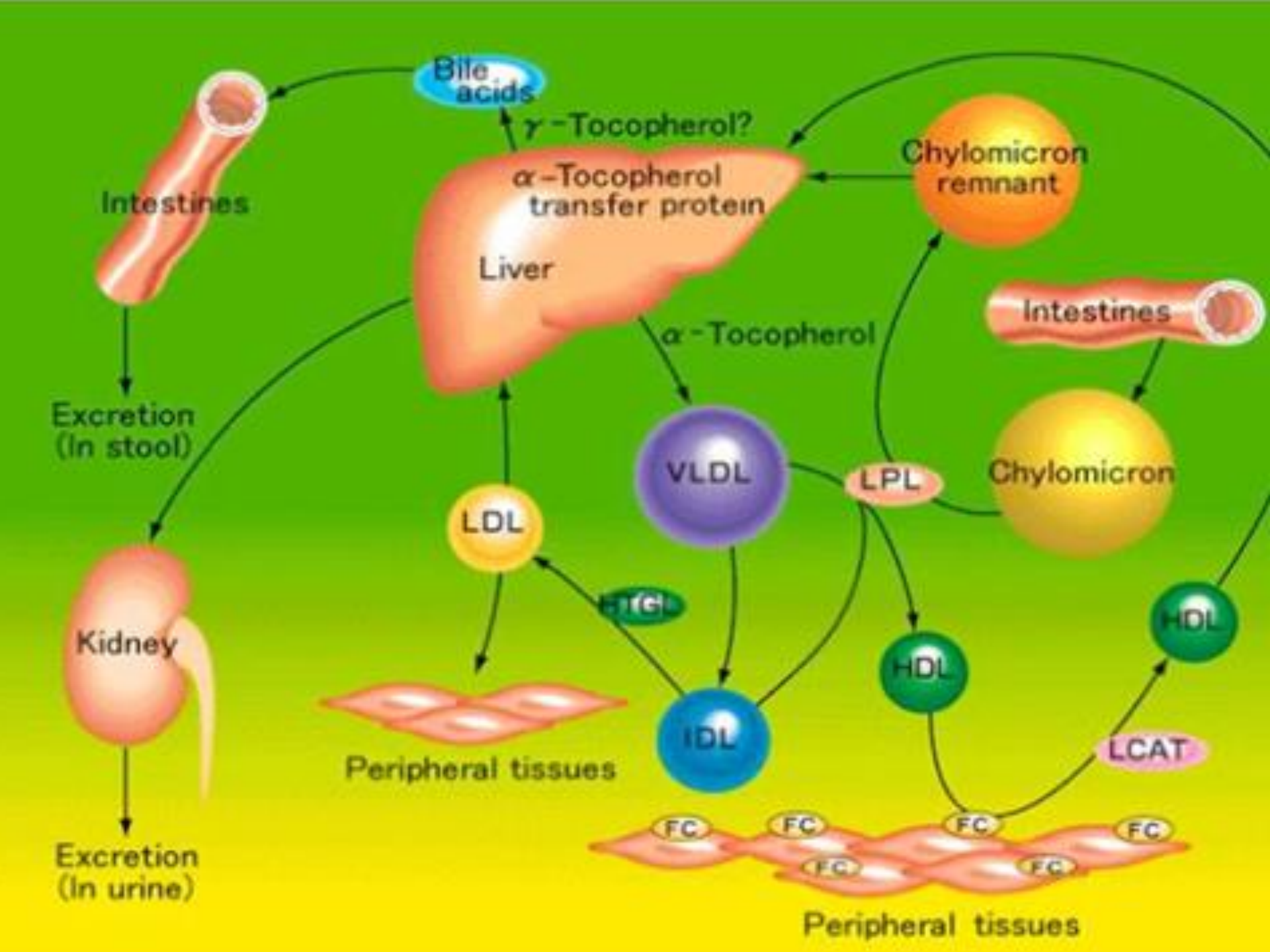
- **Chylomicrons, chylo-remnants**
- **VLDL**: 2-3 subfr
- **IDL**: 3 subfr
- **LDL**: 7 subfr
- **Lp(a)**: 3 subfr
- **HDL**: 3-10 subfr



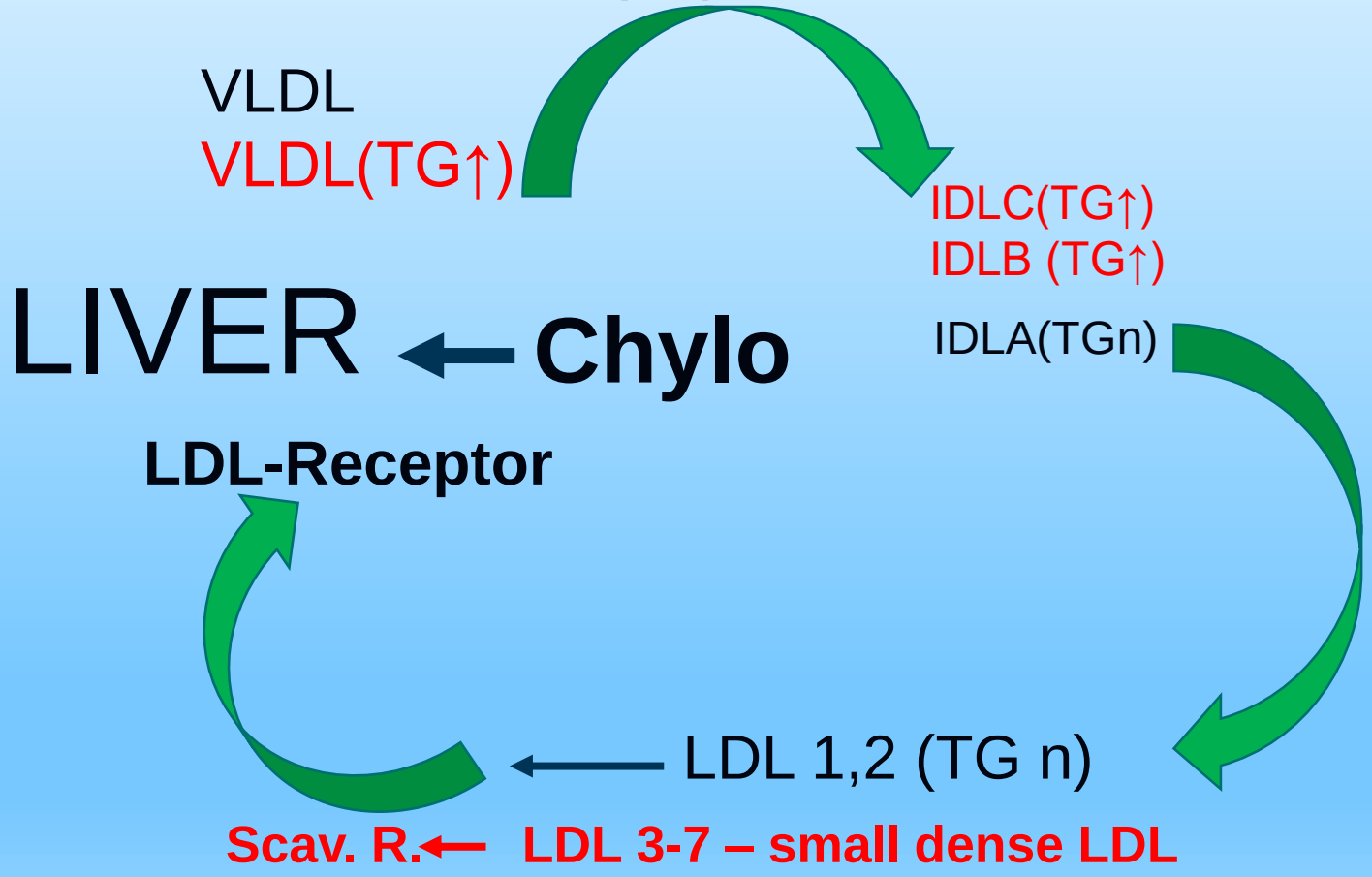
° **Different physico-chemical characteristics,**

° **different biological role**

° **different atherogenic potencial**


Bile acids
γ-Tocopherol?
α-Tocopherol transfer protein
Liver
Intestines
Chylomicron remnant
Intestines
α-Tocopherol
Excretion (In stool)
VLDL
LPL
Chylomicron
LDL
Kidney
HDL
HDL
IDL
LCAT
Peripheral tissues
FC
FC
FC
FC
FC
FC
Excretion (In urine)
Peripheral tissues

Metabolism of atherogenic lipoproteins



Small dense LDL are highly atherogenic

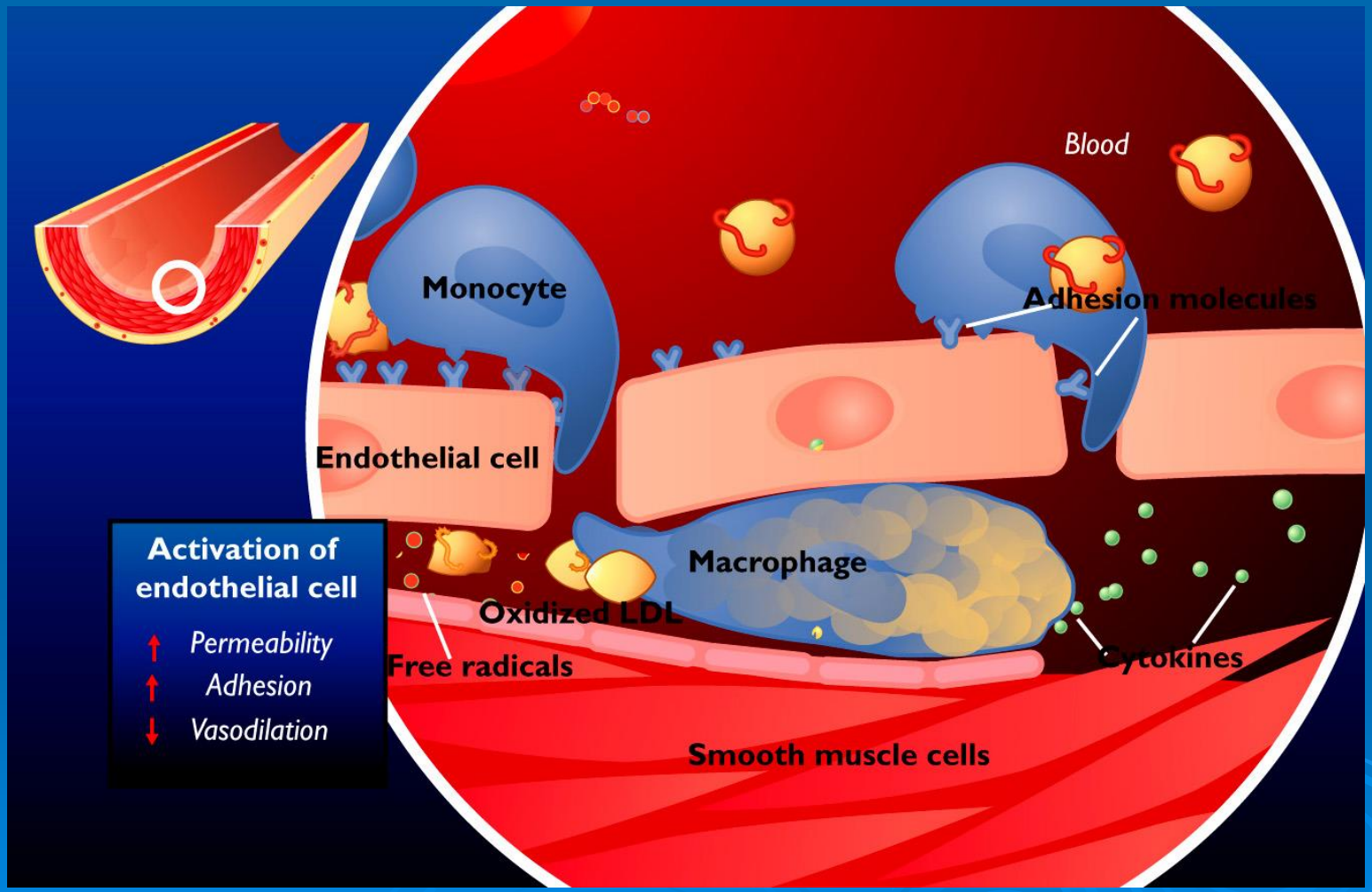
- Increased aptitude for trapping by macrophages
  configuration alterations of Apo B →
  decreased (low) recognition of LDL by LDL-receptors

* Enhancened aptitude for oxidation* and acetylation →
  oxid-LDL → release of pro-inflamatory cytokines
  → apoptosis of muscle cells & endothelial cells
  oxid-LDL → release of metalloproteinases
  → collagen degradation (cave instable angina)
  oxid-LDL → increased aptitude for trapping by macrophages
  → stimulation of foam cells formation

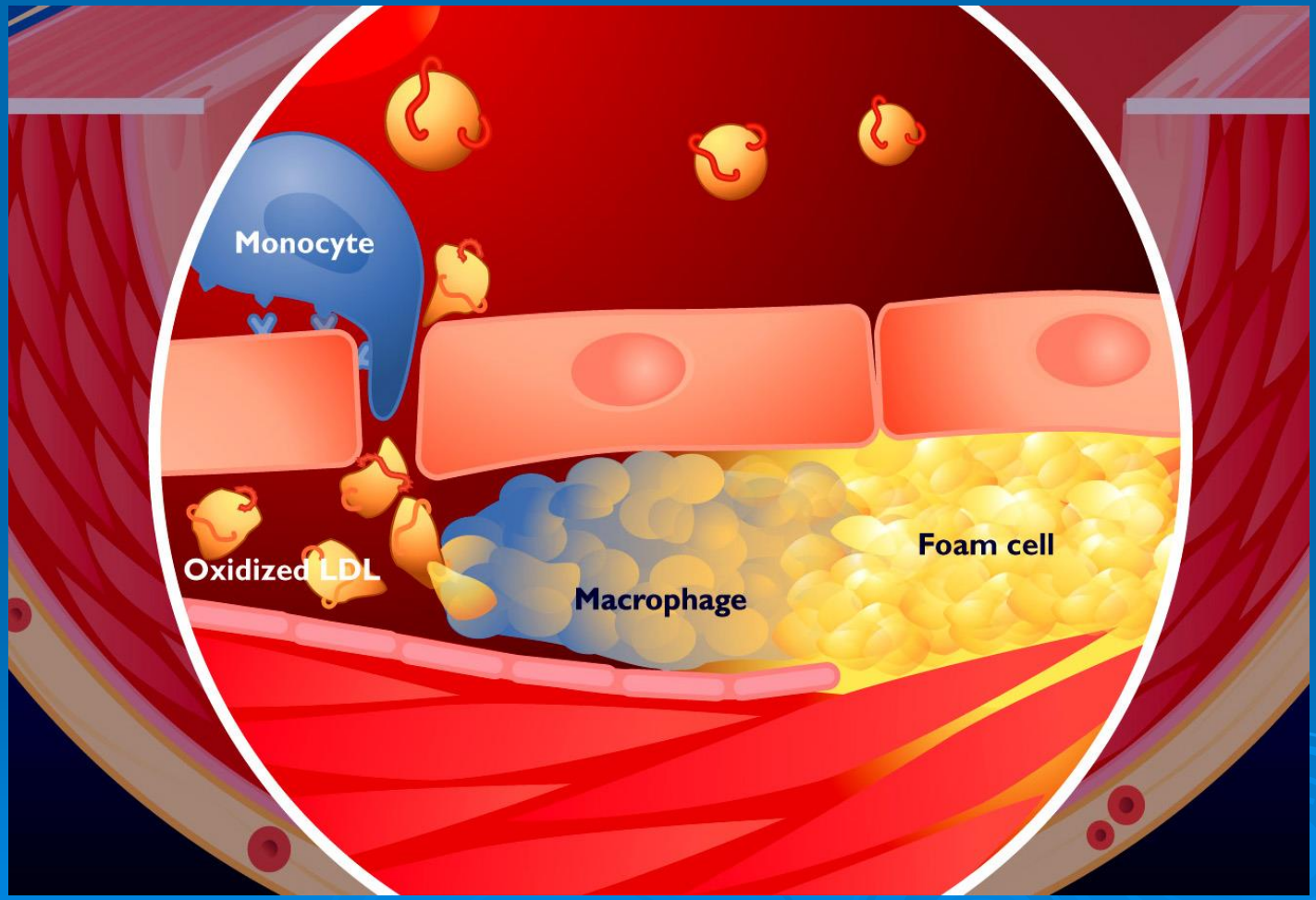
* Easier penetration into the subendothelial space →
  formation of cholesterol deposits
  and atheromatous plaques

* **Steinberg D. Lipoproteins and the patogenesis of atherosclerosis Circulation 1987; 76: 504-7**

Vascular endothelium modification in atherosclerosis



Plaque formation
Fatty streak



Small dense LDL

- **high predictive value in diagnostics of CVD:**

✓predictor of rise of coronary heart disease

✓predictor of rise of cerebro-vascular event - stroke

✓predictor of atherosclerotic impairment of arteries in all organism

in normolipemic individuals too

Literature: sdLDL predictor of cardio- and cerebro-vascular events

- Toft-Petersen AP, Tilsed HH, Aarone J et al.: Lipids in Health and Disease 2011; 10:2 1-7
- Younis NN.: Diabetes & Vascular Disease Research 2010; 7: 4 289-295
- Moore KJ, Sheedy FJ, Fisher EA.: Nat Rev Immunol 2013; 13: 709-721
- Packard CJ. Curr Opin Lipidol 2006; 17: 412-417
- Rizzo M, Pernice V, Frasheri A et al.: Clin Endocrinol 2009; 70: 870-875
- Paulsen K, Schulte D, Türck K et al.: Diabetol Stoffwechs 2016; 11: 83
- Shen H, Xu L, Lu J et al.: Chinese population. Lipids Health Dis. 2015, 14:137, 1-6
- Hoogeveen RC, Gaubatz JW, Sun W.et al.: Atheroscler Thromb VascBiol 2014; 34: 1069-1077
- Zaki-Khalil RMA, Al Azab DAM, Akl AO: Alexandria J Med 2017; 53: 299-305

- Ai M, Otokozawa S, Asztalos BF et al.: Clin Chem 2010 56: 967-976
- Srisawasdi P, Chaloeysup S, Teerajetgul Y et al.: Am J Clin Pathol 2011:
- Jacobson TA, Miller M, Hirayama S, Miida T. Small dense LDL. Clin Chim Acta 2012: 414: 215-224
- Fan J, LiuY, Yin S et al.: Nutr Metab 2019: 16: 7
- Shiffman D, Louie JZ, Caulfield MP et al.:Atherosclerosis 2017: 263: 297-
- Hirayama S, Miida T. : Clin Chim Acta 2012: 414: 215-114
- Nouze T, Michishita I, Ishibashi Y et al.: J Atheroscler Thromb 2007: 14: 202-207
- Schaefer EJ. Clin Ther 2007: 29: 763-77 136: 20-29
- Wolska A, Remaley AT. Clin Lab News 2017: 1: 17-20
- Matsura Y, Kanter JE, Bornfeldt KE: Arterioscler Thronb Vasc Biol 2019: 39: E1-E9
- Sampson M, Wolska A, Warnick R. et al.: Clin Chem 2021: 67: 987-997

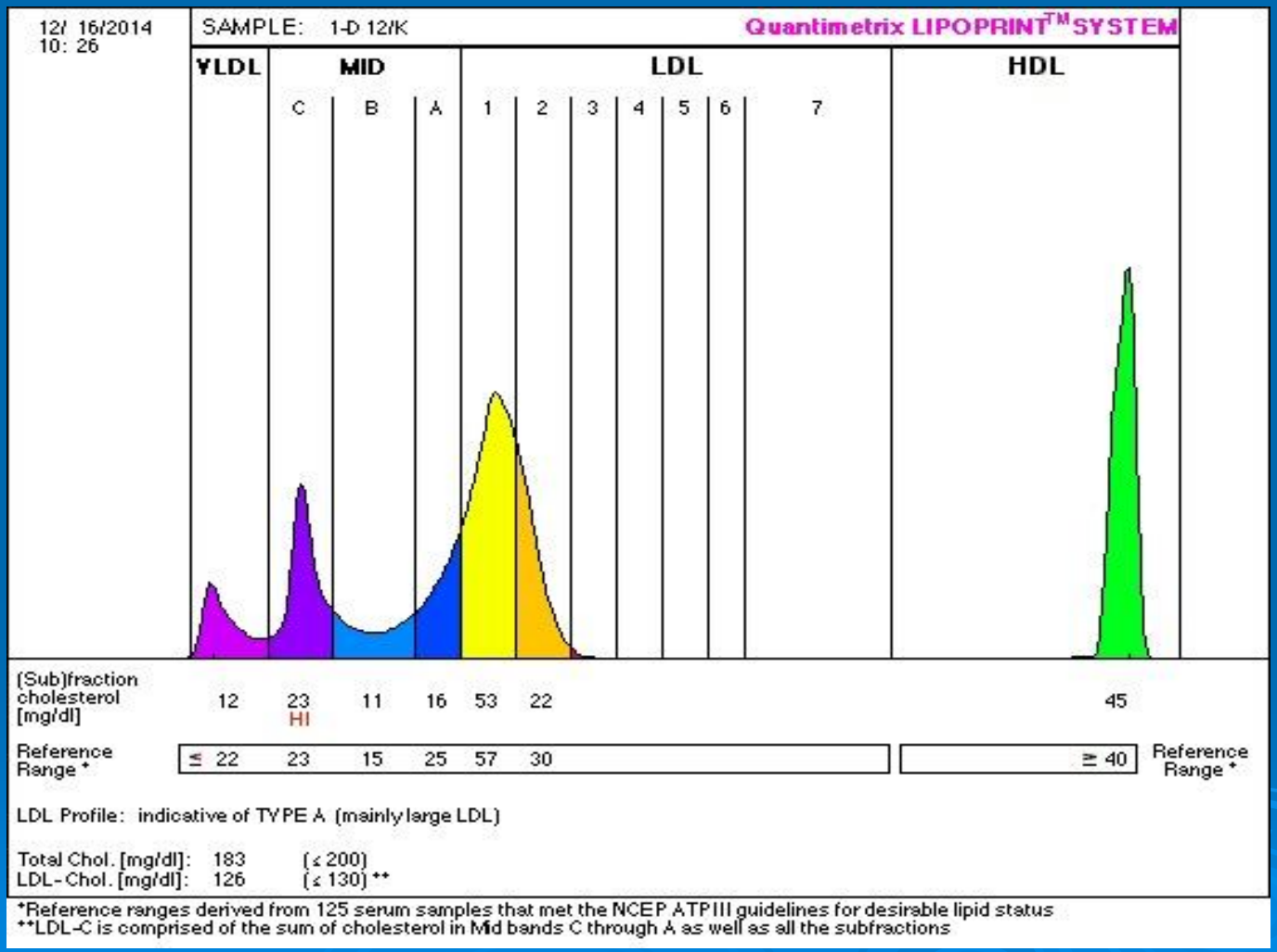
Lipoprint LDL System

Electrophoretic analysis of plasma lipoproteins on polyacrylamide gel (PAG) according to the size of lipoprotein particles

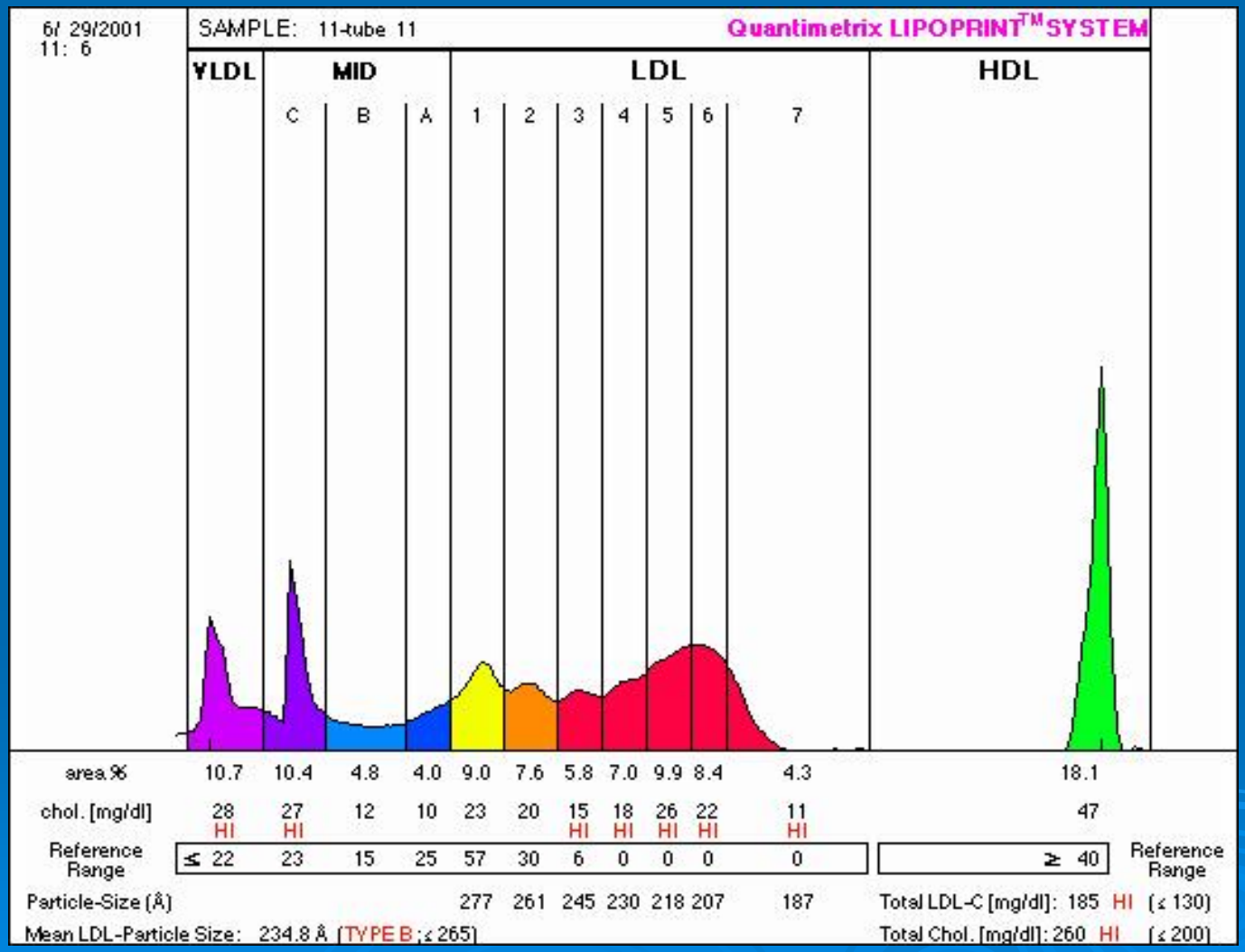
Milestone in laboratory analysis and clinical diagnostics, it helps to analyse up to 14 lipoprotein entities: i.e. lipoprotein families and lipoprotein subfractions

VLDL, IDL 1 - 3, LDL 1 - 7, HDL 1 - 10

Nonatherogenic normolipemia



Atherogenic dyslipidemia

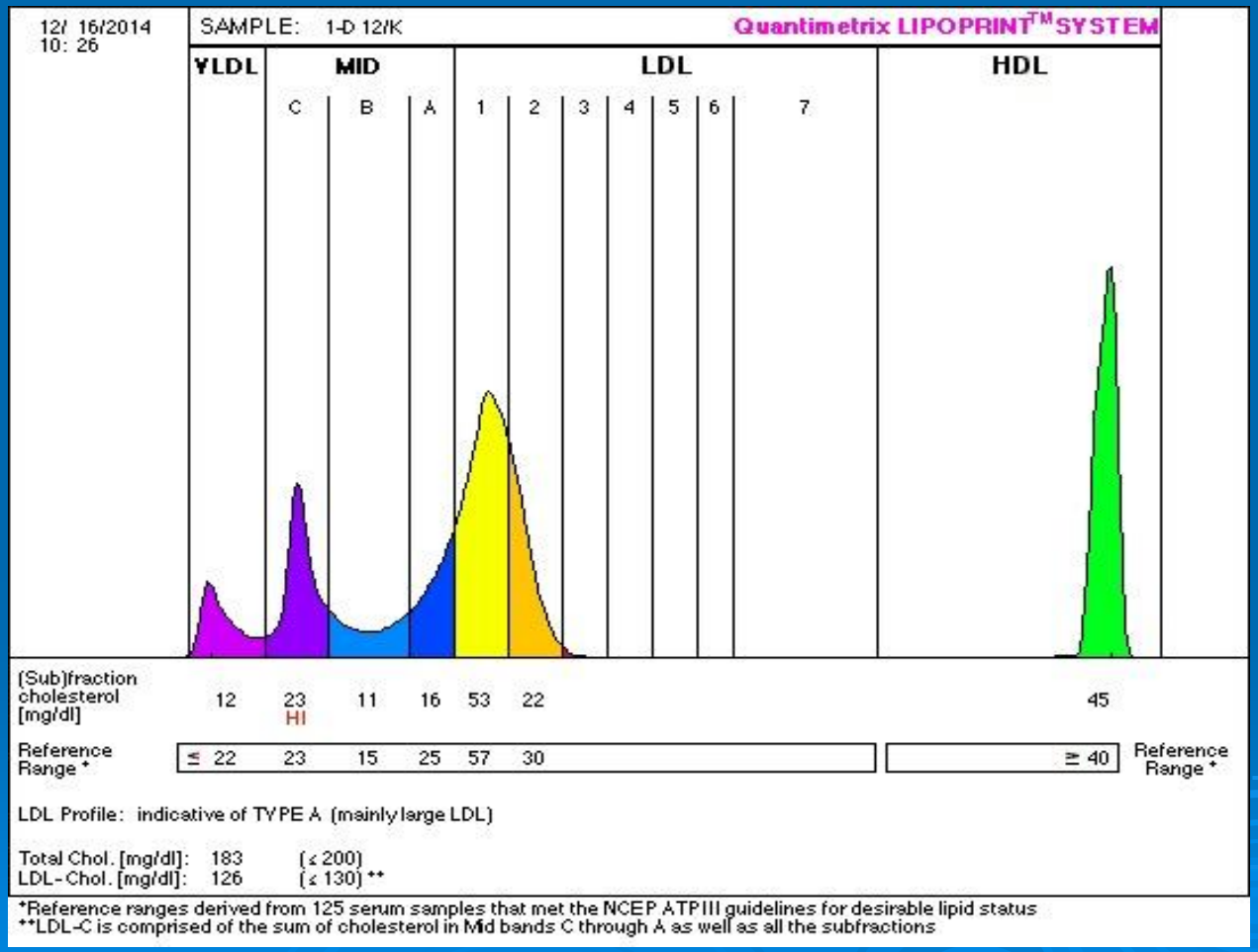


Clinical study
Identification of plasma Lp- profile

* **Control group** – healthy probands
* **Arterial Hypertension**
* **Coronary Heart Disease**
* **Peripheral Artery Diesase**
* **Stroke**
* **Hyper-β-lipoproteinemia LDL1,2**
* **Gilbert syndrome** (hypercholesterolemia, normolipemia)

- ✓ ***Oravec S, Mikl J, Gruber K, Dostal E**.: in Lipoproteins, Role in Health and Diseases ed.: S Frank G Kostner, InTech open science Rijeka, 2014 p.74-94*
- ✓ ***Oravec S, Gruber K, Dukat A, Gavorník, P,Gašpar L, Dostal E**.: in Lipoproteins from Bench to Bedside ed.: G Kostner, I Chennamesetty InTech open science Rijeka, 2015 p.87-111*
- ✓ ***Očadlik I, Hlinšťaková S, Oravec S.:** Neuroendocrinol Lett 2011; 32(3): 360-364*

Control group - Nonatherogenic Normolipemia

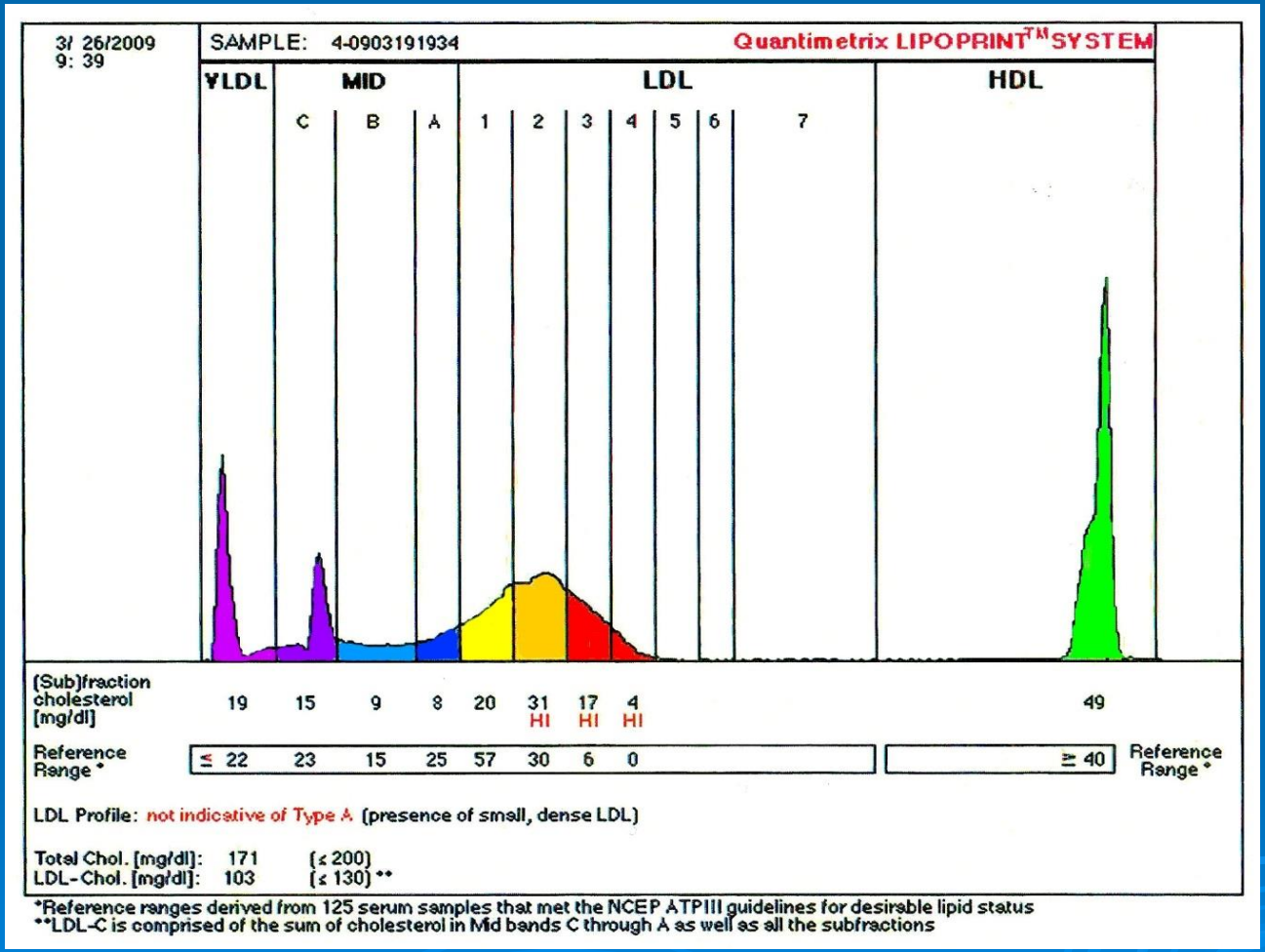


Lipids and lipoproteins in the Control group (n= 165)

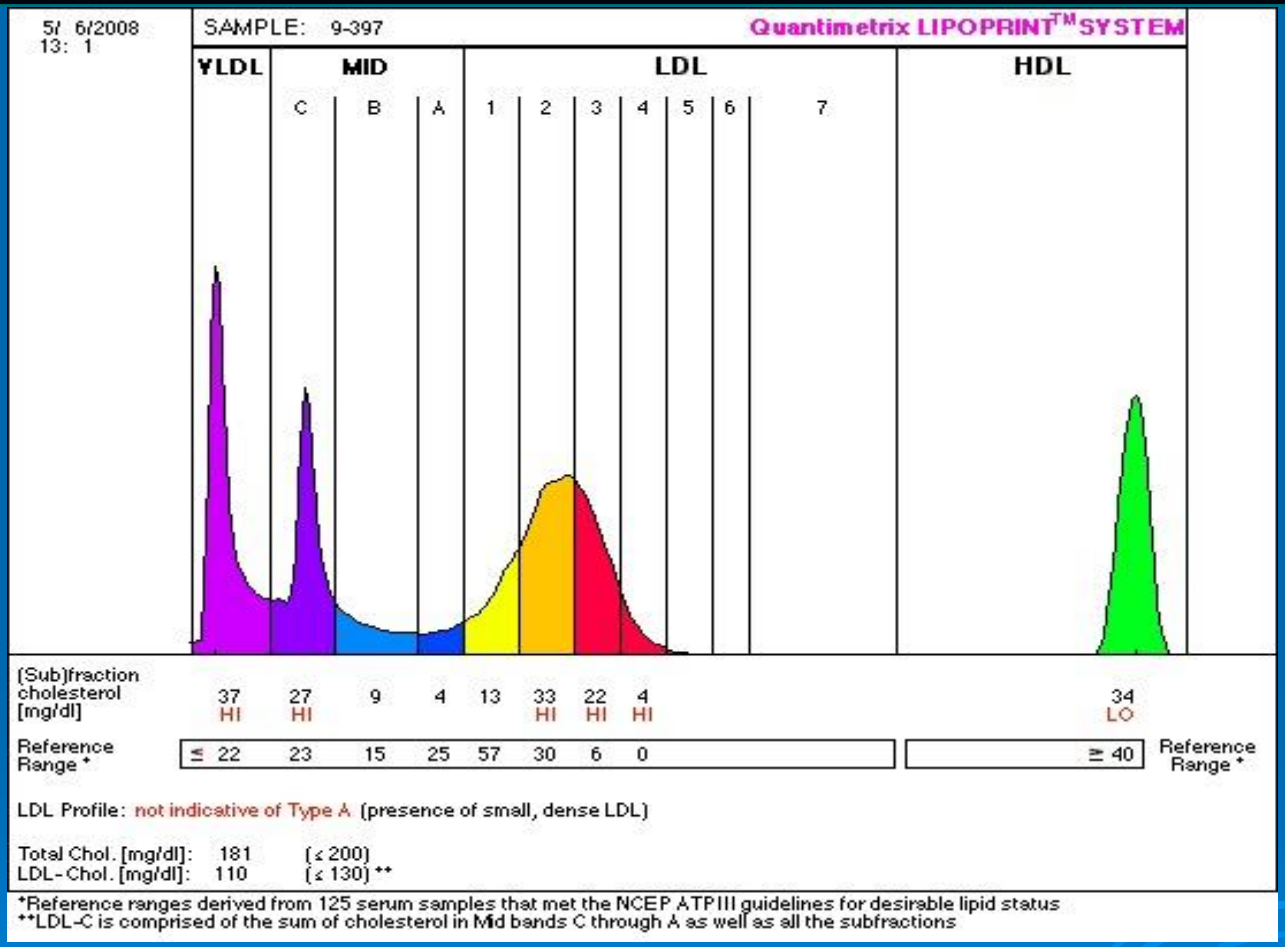
	Chol (mmol/l SD	TAG	VLDL	LDL1,2	LDL3-7	LDL	HDL	Score
Contr (n=165)	**4.31** ±0.62	**1.16** ±0.39	**0.62** ±0.16	**1.30** ±0.24	**0.04** ±0.04	**2.34** ±0.54	**1.33** ±0.32	**36.1** ±20.6
Contr	**4.31** ±0.62	**1.12** ±0.38	**0.62** ±0.16	**1.31** ±0.24	**0.03** ±0.04	**2.33** ±0.54	**1.33** ±0.32	**38.1** ±19.6
(nonatherogen. profile n= 155)								
Contr	**4.37** ±1.14	**1.63** ±1.34	**0.72** ±0.14	**1.22** ±0.15	**0.25** ±0.31	**2.37** ±0.34	**1.27** ±0.36	**5.3** ± 2.0
(atherogen. profile n= 10)								
nonathero vs. athero		p<0.001			p< 0.0001			p< 0.0001

Control - Atherogen. profile 6.6 %

Atherogenic Normolipemia



Arterial Hypertension



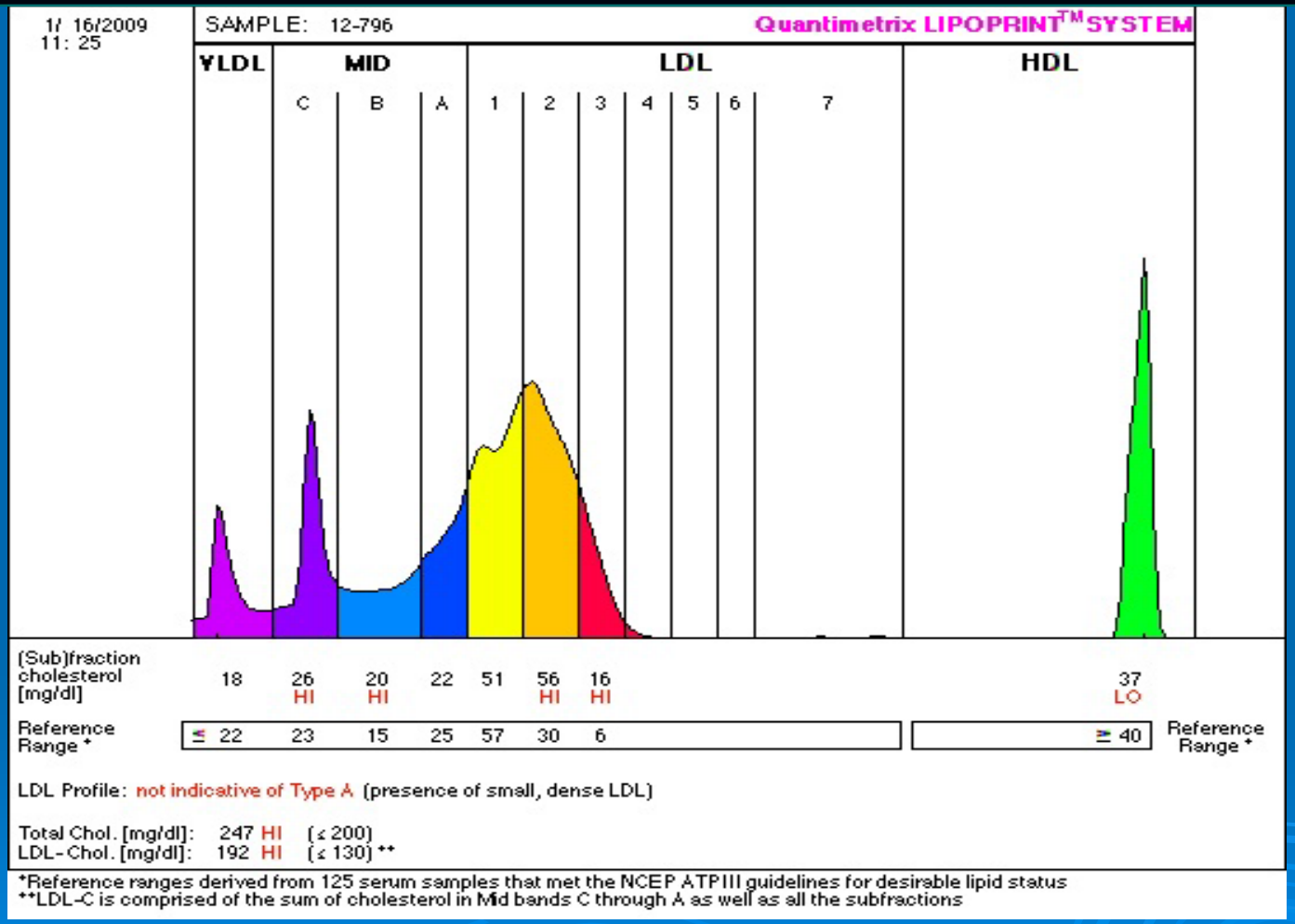
- increased TG, VLDL a VLDL-remnants
- small dense LDL are present in LDL 3,4 subfractions

Lipids and lipoproteins in Arterial Hypertension (n= 107)

	Chol (mmol/l SD	TAG	VLDL	LDL1,2	LDL3-7	LDL	HDL	Score
Contr (n=165)	**4.31** ±0.62	**1.16** ±0.39	**0.62** ±0.16	**1.30** ±0.25	**0.04** ±0.04	**2.34** ±0.54	**1.33** ±0.32	**36.1** ± 20.6
AH (nonatherogen.profile n= 23)	**5.32** ±0.98	**1.56** ±0.55	**0.84** ±0.31	**1.78** ±0.44	**0.03** ±0.04	**3.02** ±0.71	**1.49** ±0.34	**24.2** ±13.6
AH (atherogen profile n= 84)	**5.15** ±1.14	**2.48** ±1.34	**1.01** ±0.35	**1.27** ±0.58	**0.42** ±0.31	**2.99** ±0.96	**1.18** ±0.34	**5.1** ± 2.0
nonathero vs. athero		p<0.001		p<0.01	p< 0.0001		p< 0.001	p< 0.0001

AH - Atherogen. profile 78.5 %

Coronary Heart Disease – CHD



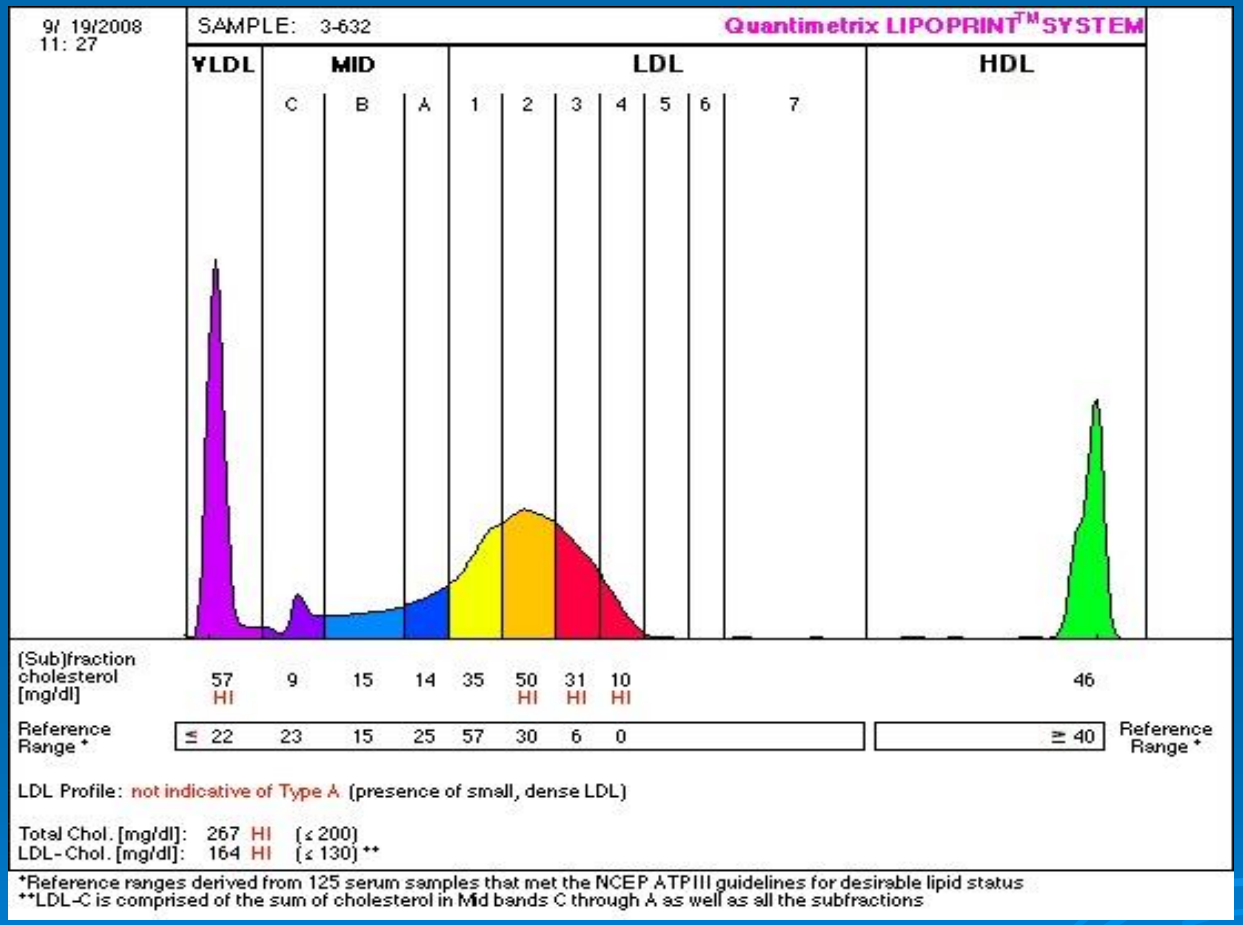
Hypercholesterolemia, increased conc. of atherogenic sdLDL

Lipids and lipoproteins in CHD (n= 104)

	Chol (mmol/l±SD)	TAG	VLDL	LDL1,2	LDL3-7	LDL	HDL	Score
Contr (n=165)	**4.31** ±0.62	**1.16** ±0.39	**0.62** ±0.16	**1.30** ±0.25	**0.04** ±0.04	**2.34** ±0.54	**1.33** ±0.32	**36.1** ± 20.6
CHD (nonatherogen. profile n=19)	**5.24** ±0.99	**1.44** ±0.50	**0.82** ±0.27	**1.73** ±0.49	**0.13** ±0.06	**3.11** ±0.80	**1.29** ±0.31	**12.7** ±5.1
CHD (atherogen. profile n= 85)	**5.27** ±1.19	**2.63** ±2.03	**1.02** ±0.47	**1.47** ±0.46	**0.48** ±0.35	**3.05** ±0.86	**1.16** ±0.28	**4.0** ±3.5
Nonathero vs. athero profile		p<0.01		p<0.05	p< 0.0001			p< 0.0001

CHD - Atherogenic Lp-profile 81.7%

Peripheral Artery Disease - PAD



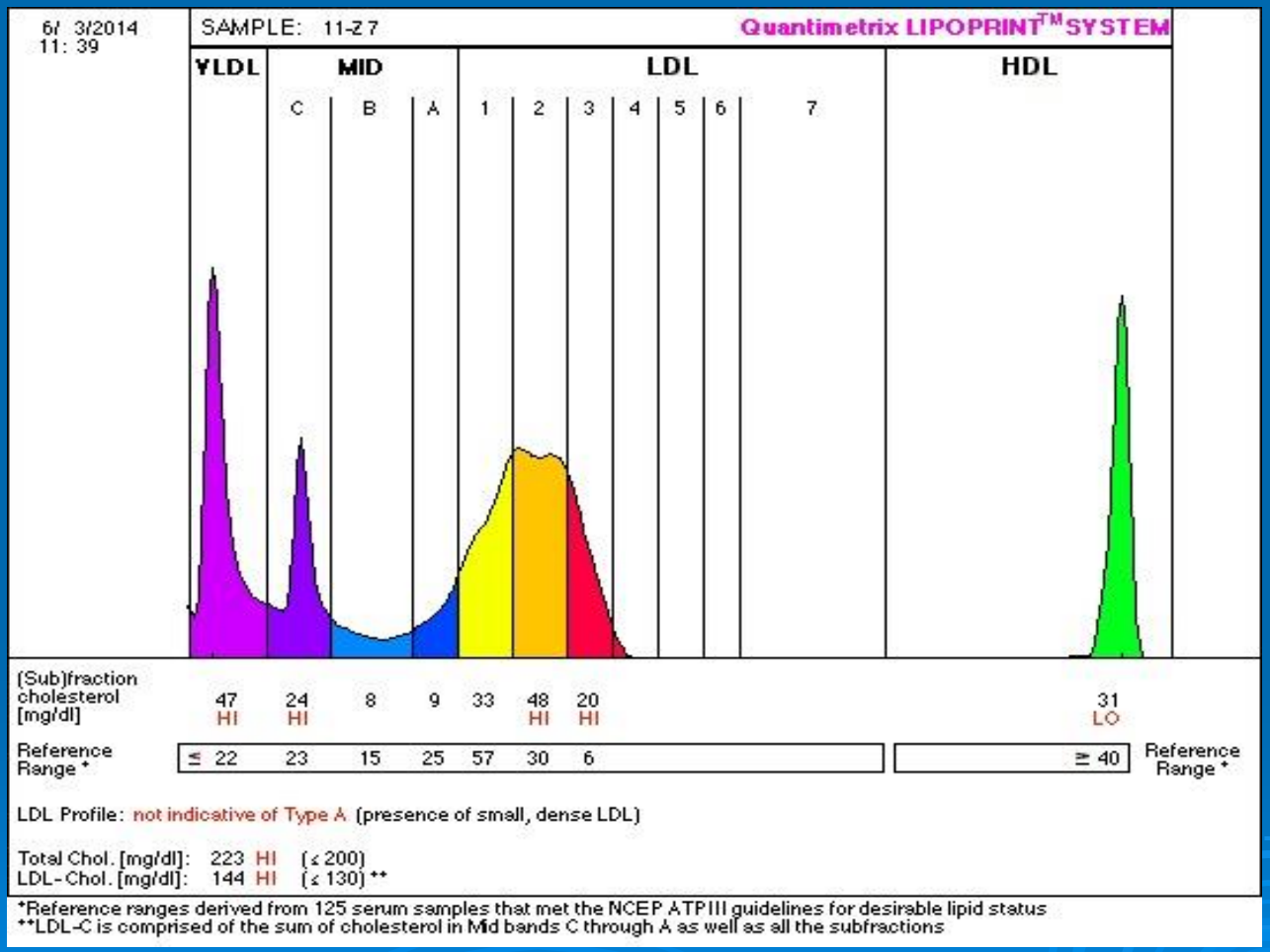
combined HLP with a high **c**onc. of VLDL and small dense LDL (LDL3,4 subfractions)

Lipids and lipoproteins in PAD (n=100)

	Chol (mmol/l±SD)	TAG	VLDL	LDL1,2	LDL3-7	LDL	HDL	Score
Contr (n=165)	**4.31** ±0.62	**1.16** ±0.39	**0.62** ±0.16	**1.30** ±0.25	**0.04** ±0.04	**2.34** ±0.54	**1.33** ±0.32	**36.1** ± 20.6
PAD (nonatherogen.profile n= 20)	**5.37** ±0.95	**1.81** ±0.51	**0.86** ±0.26	**1.82** ±0.54	**0.10** ±0.03	**3.18** ±0.82	**1.33** ±0.29	**17.4** ± 6.5
PAD (**atherogen. profile** n= 80)	**5.28** ±1.28	**2.31** ±1.18	**0.98** ±0.39	**1.52** ±0.50	**0.46** ±0.34	**3.09** ±0.99	**1.18** ±0.32	**4.6** ± 4.0
Neathero vs. athero		p<0,01		p<0,05	p< 0,0001			p< 0,0001

PAD – Atherogenic Lp-profile 80 %

Stroke



combined HLP with high conc. VLDL and sdLDL (LDL 3,4 subfractions)

Lipids, lipoproteins in patients surv. stroke (n=55)

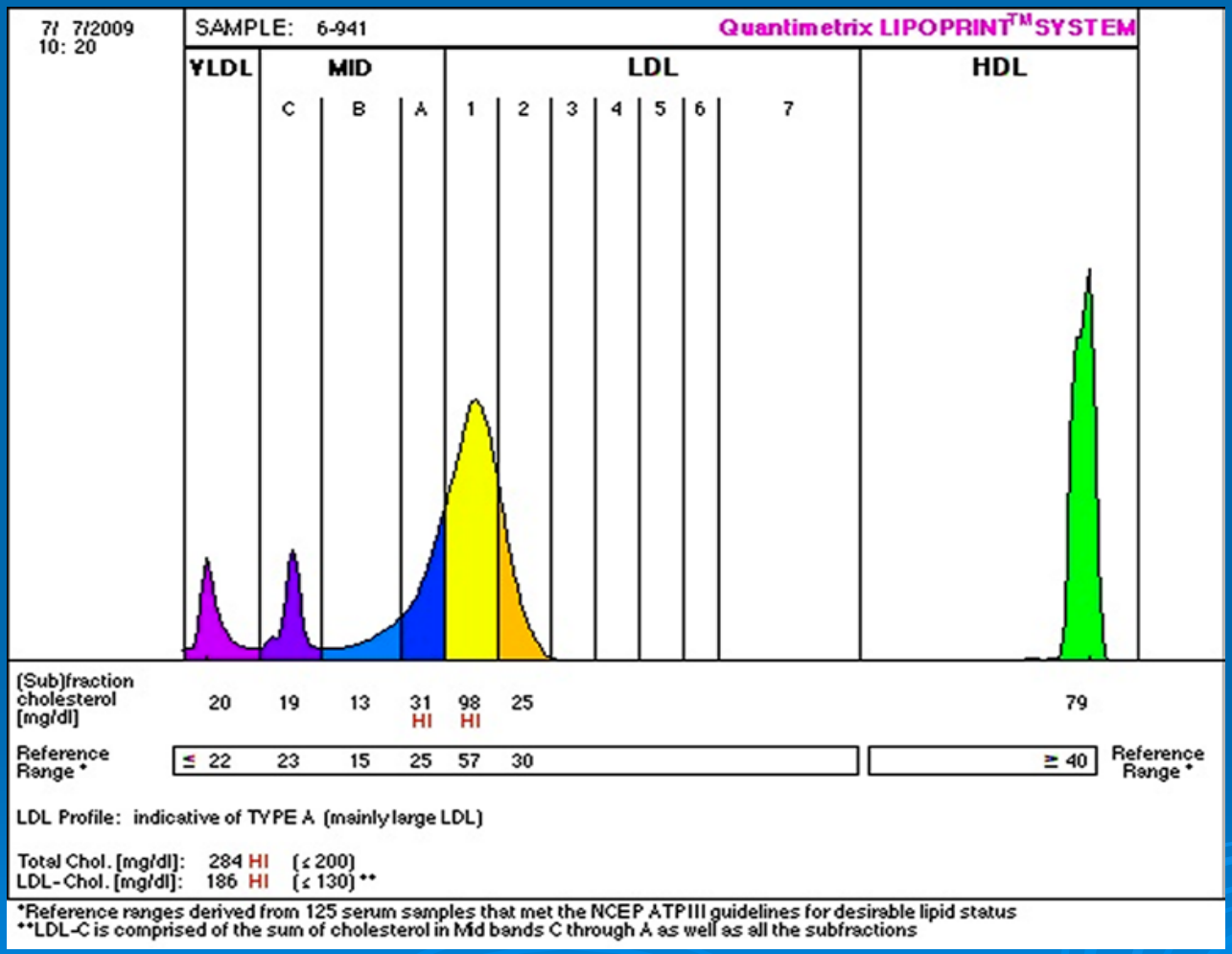
	Chol (mmol/l±SD)	TAG	VLDL	LDL1,2	LDL3-7	LDL	HDL	Score	LDL1	LDL2
Contr (n=165)	**4.31** ±0.62	**1.16** ±0.39	**0.62** ±0.16	**1.30** ±0.25	**0.04** ±0.04	**2.34** ±0.54	**1.33** ±0.32	**36.1** ± 20.6	**0.89** ± 0.28	**0.41** ± 0.21
Stroke	**5.54** ±1.30	**1.70** ±0.44	**0.93** ±0.14	**2.19** ±0.86	**0.14** ±0.07	**3.30** ±1.01	**1.31** ±0.35	**13.74** ±1.36	**1.22** ±0.45	**0.92** ±0.44
(nonatherogen. profile n= 8)										
Stroke	**5.14** ±1.11	**2.29** ±0.94	**1.11** ±0.37	**1.48** ±0.41	**0.31** ±0.23	**2.86** ±0.72	**1.06** ±0.29	**5.33** ±3.32	**0.72** ±0.26	**0.76** ±0.26
(atherogen.profel n = 47)										

Nonathero vs.athero profile

p<0.002 p<0.001 p<0.001 p< 0.0001 p< 0.0001

Stroke – Atherogen. Lp-profile 85.5 %

Hyper-β-lipoproteinemia LDL1,2



- hypercholesterolemia without small dense LDL

✓ *Oravec S and co.. Neuroendocrinol Lett 2011; 32(3) 322-27*

Lipids and lipoproteins in hyper-β-lipoproteinemia LDL1,2 (n=145)

	Chol	TAG	VLDL	LDL1	LDL2	LDL3-7	LDL	HDL	Score
	(mmol/l±SD)								
Contr	**4.31**	**1.16**	**0.62**	**0.86**	**0.39**	**0.04**	**2.34**	**1.33**	**36.1**
(n=165)	±0.62	±0.39	±0.16	±0.26	±0.22	±0.04	±0.54	±0.32	± 20.6
HβLP	**6.71**	**1.29**	**0.74**	**1.68**	**0.52**	**0.01**	**4.09**	**1.88**	**76.0**
	±0.90	±0.49	±0.21	±0.36	±0.29	±0.03	±0.82	±0.29	± 6.5

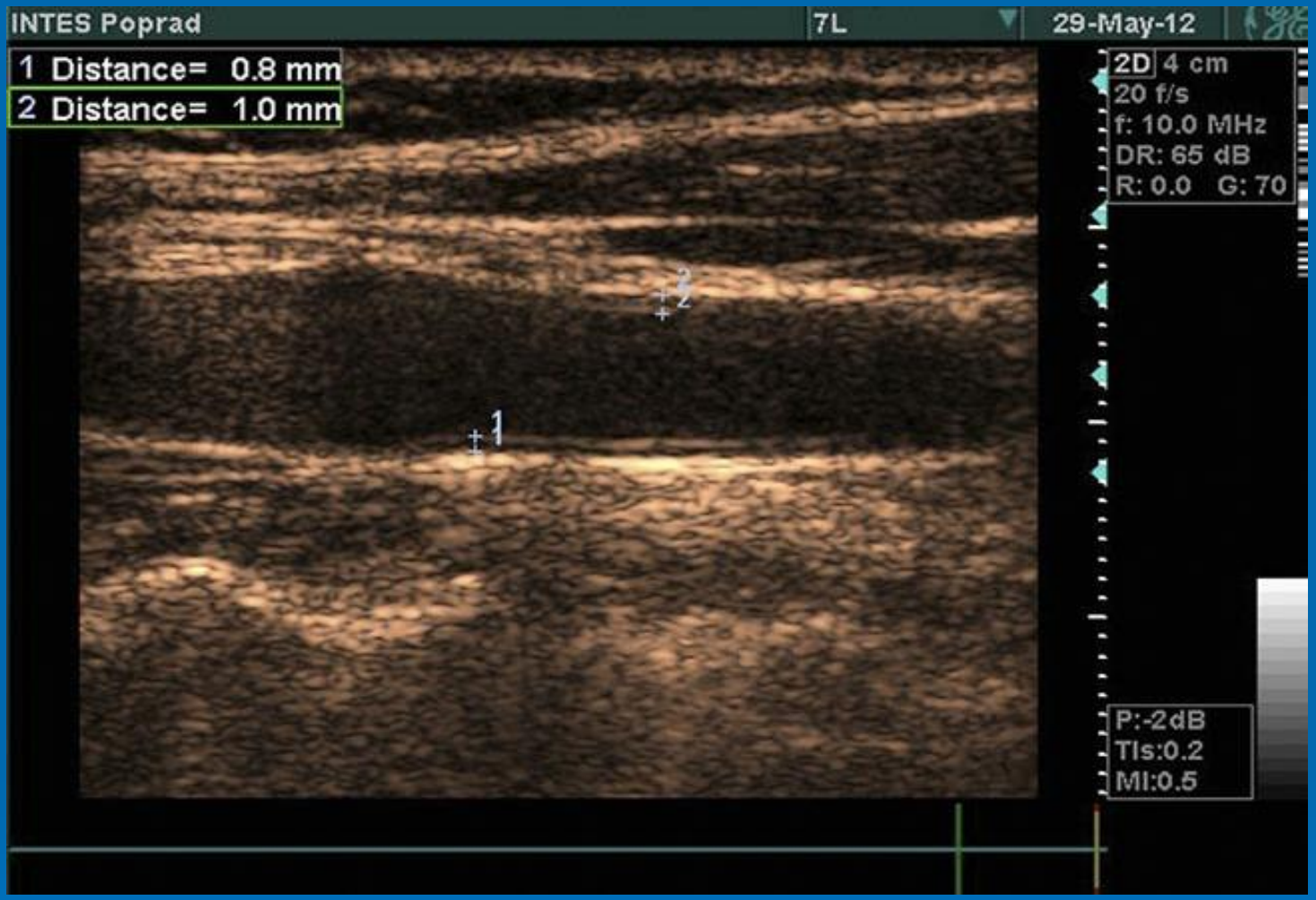
(nonatherogen. profile n= 145)

Contr vs. HβLP

p<0.0001 p<0.0001 p< 0.0001 p< 0.0001

p< 0.001

HβLP Nonatherogenic Lp-profile

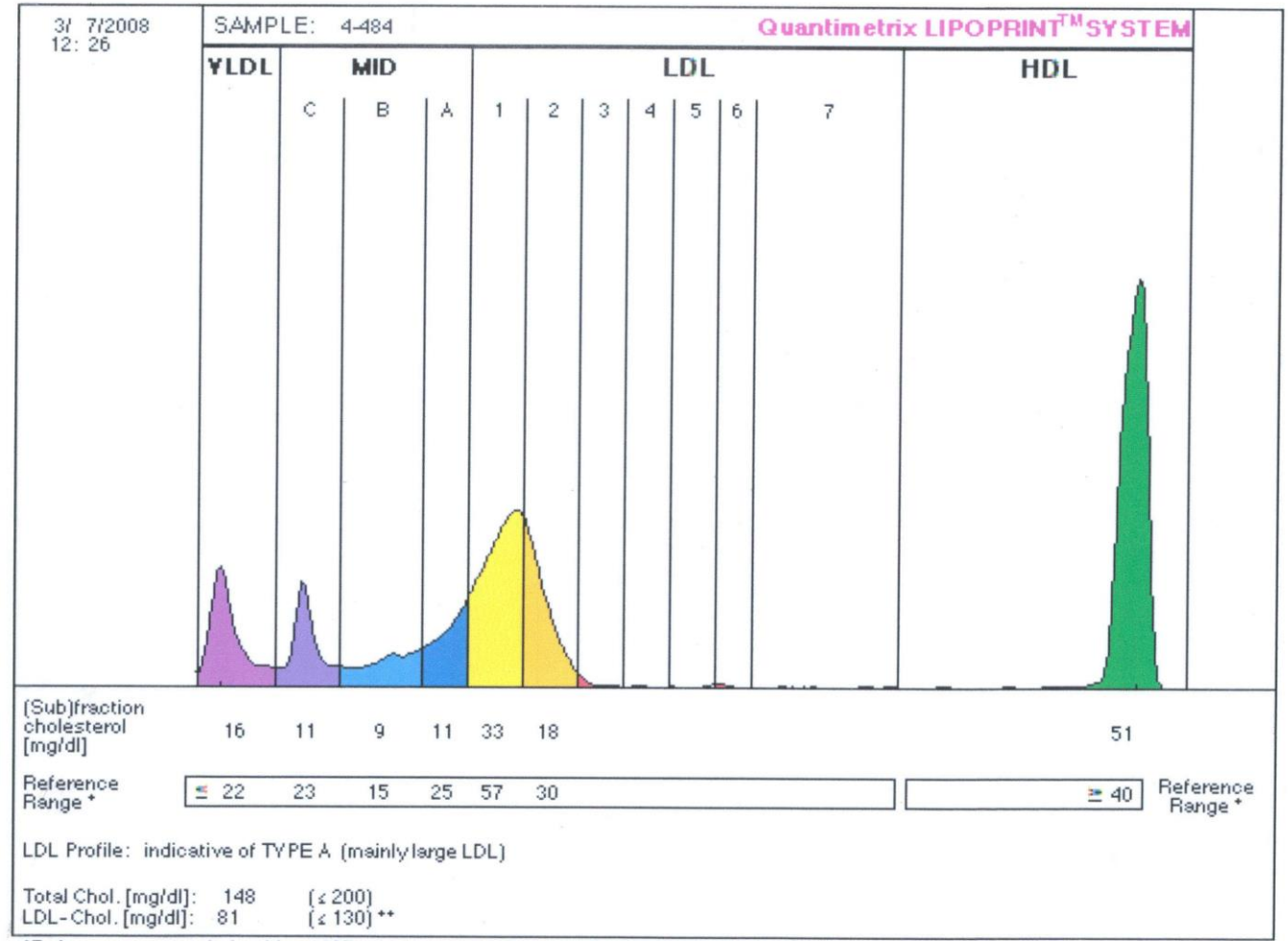


Discret changes in IMT in persons with **nonatherog. hyper-β-lipoproteinemia** do not correlate with an interpretation of hypercholesterolemia as an atherogenic risk factor.

(sono image: Vacula I. I.Med.Clinic MedFaculty UK)

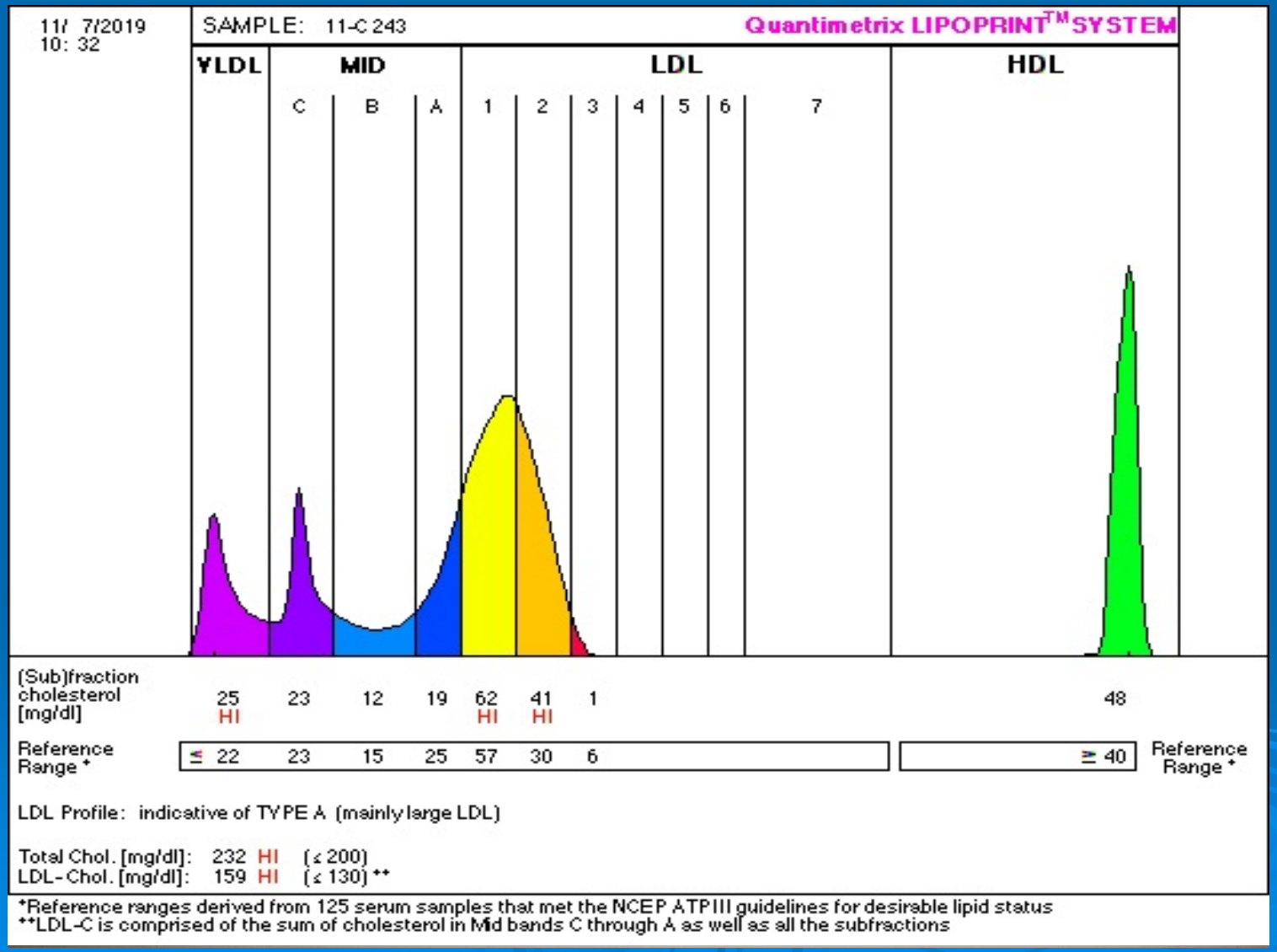
Gilbert syndrome

Normolipemia without sdLDL, high HDL



Gilbert syndrome

Hypercholesterolemia without sdLDL, high HDL



Lipids and lipoproteins and Bilirubin Gilbert syndrome (n=40)

	Chol (mmol/l±SD)	TAG	VLDL	IDL	LDL1,2	LDL3-7	LDL	HDL	Bi.t. (µmol/l±SD)	Bi.nk.
Contr. (n=165)	**4.31** ±0.62	**1.16** ±0.39	**0.62** ±0.16	**0.33** ±0.12	**1.30** ±0.25	**0.04** ±0.04	**2.34** ±0.54	**1.33** ±0.32	**9.73** ± 4.60	**4.89** ±8.26
Gil.sy. (n= 40)	**4.49** ±0.82	**1.01** ±0.47	**0.51** ±0.19	**0.93** ±0.25	**1.72** ±0.47	**0.01** ±0.02	**2.66** ±0.60	**1.32** ±0.30	**25.91** ±12.8	**16.23** ±10.05
GS vs.Kontr						p< 0,05			p< 0,001	p< 0,001

- **Gilbert sy** **Nonatherogenic Lp profile**

Biochemical parameters Gilbert syndrome (n=40)

	AST (ukat/l±SD)	ALT	GMT	ALP	Bi. tot. (µmol/l±SD)	Bi. konj.	Bi. nonkonj.
Contr. (n=165)	**0.39** ±0.08	**0.40** ±0.12	**0.42** ±0.25	**0.99** ±0.26	**9.73** ± 4.60	**8.84** ±3.11	**4.89** ±8.26
Gil.sy. (n= 40)	**0.41** ±0.14	**0.46** ±0.27	**0.35** ±0.19	**0.92** ±0.26	**25.91** ±12.80	**6.98** ±1.44	**16.23** ±10.05
					p< 0.001		p< 0.001

Conclusions

Small dense LDL (sdLDL):

° a regular part of Lp-spectrum in human plasma

° Identification of sdLDL in diagnostics of CVD
- principial contribution

° **lipoprotein profile: phenotype B / phenotype A**

° Patients with CVD: **High presence of sdLDL in Lp-profile**

atherogenic lipoprotein profile present in 80 – 86 %

° in Hypercholesterolemia:

Hyper-β-lipoproteinemia LDL1,2

Gilbert syndrome

- sdLDL - present in traces only

nonatherogenic lipoprotein profile, phenotype A

° **Confirmation of existence**

nonatherogenic hypercholesterolemia

and atherogenic normolipemia

in clinically healthy individuals


THANK YOU
FOR YOUR
ATTENTION