

Classifications of Lipoproteins

1. Operational Classification

Physico-chemical properties as differentiating criteria

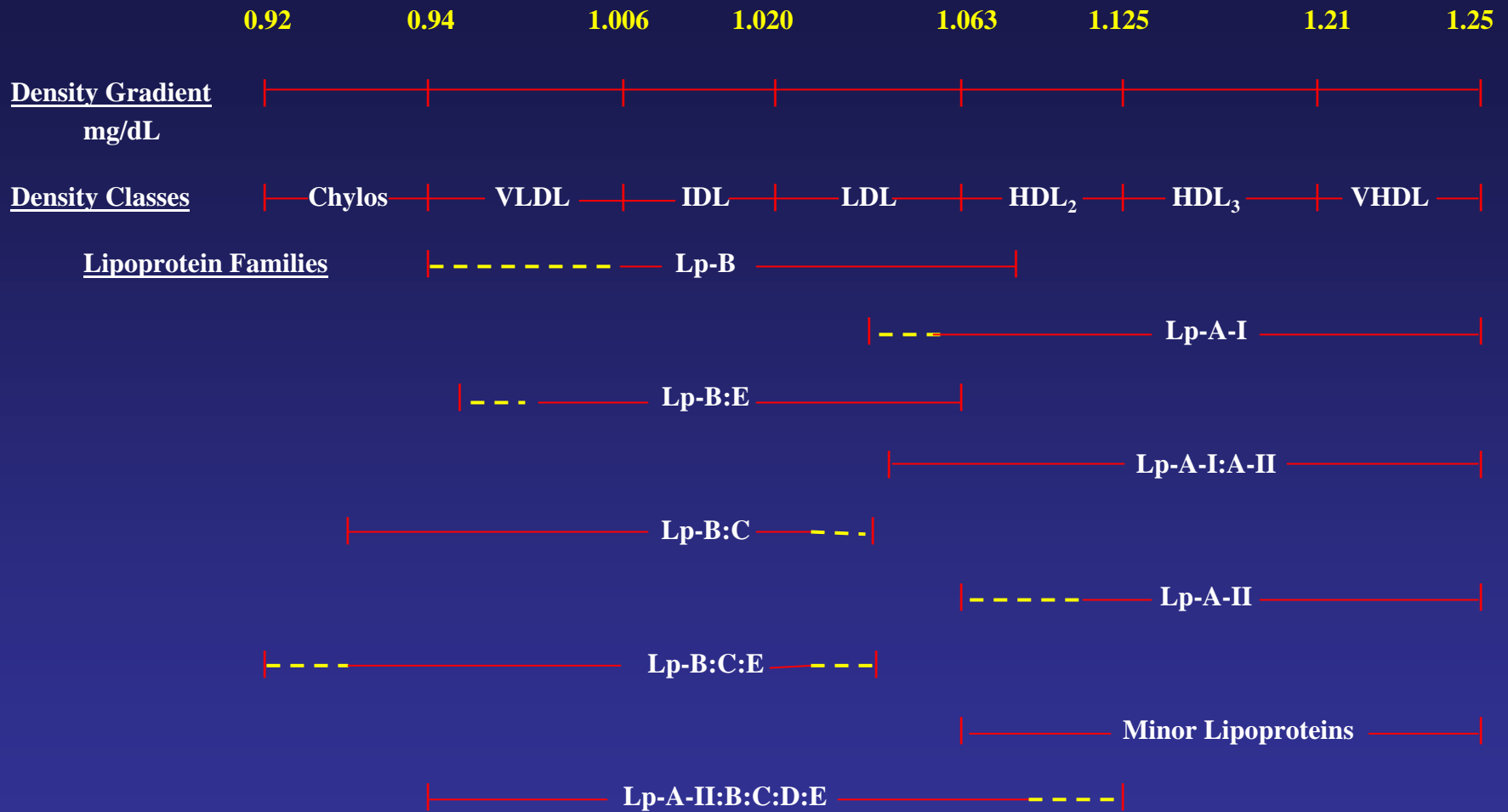
- a) Electric charge
- b) Density
- c) Size

2. Chemical Classification

Apolipoprotein composition as the differentiating criterion

Characteristics of Apolipoproteins

1. Determinants of structural integrity and specific metabolic and functional properties of lipoproteins.
 2. Unique chemical markers for identifying, characterizing and classifying lipoproteins.
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Polydispersity of ApoA- and ApoB-containing Lipoproteins

Each apoA- and apoB-containing lipoprotein family is a polydisperse system of particles heterogeneous with respect to density, size and lipid/protein ratios but homogeneous with respect to qualitative apolipoprotein composition.

Percent Lipid Composition of ApoA-containing Lipoprotein Particles From Normolipidemic Subjects *

Lipoprotein Families	Triglycerides	Cholesterol Esters	Free Cholesterol	Phospholipids
	%			
LpA-II (n = 5)	0.3 ± 0.5	12.3 ± 3.9	4.3 ± 2.3	82.4 ± 4.8
LpA-I:A-II (n = 14)	3.4 ± 1.5	32.3 ± 4.5	5.9 ± 1.1	58.2 ± 5.2
LpA-I (n = 14)	2.8 ± 1.6	24.2 ± 6.7	6.1 ± 1.9	66.7 ± 8.4

* Mean ± SD percentages of the total lipid mass

Number of subjects in parentheses

(Bekaert et al. Pediatr Res. 1991;29:315-321)

Percent Apolipoprotein Composition of ApoA-containing Lipoprotein Particles From Normolipidemic Subjects *

Lipoprotein Families	Apolipoproteins					
	A-I	A-II	C-II	C-III	D	E
			%			
LpA-II (n = 5)	ND ^a	77.2 ± 6.8	ND	ND	22.8 ± 6.8	ND
LpA-I:A-II (n = 14)	51.1 ± 3.7	35.1 ± 4.7	0.9 ± 0.4	2.4 ± 1.7	6.2 ± 1.7	4.2 ± 2.8
LpA-I (n = 14)	88.7 ± 6.5	ND	0.6 ± 0.6	2.3 ± 2.5	5.7 ± 3.4	2.3 ± 2.5

* Mean ± SD percentages of the total apolipoprotein mass

Number of subjects in parentheses

^a ND = not detected by electroimmunoassay

(Bekaert et al. Pediatr Res. 1991;29:315-321)

**Percent Lipid Composition of Simple
ApoB-containing Lipoprotein Families
VLDL and LDL**

Density Class	Triglycerides	Cholesterol	Cholesterol Esters
		%	
<u>Lp-B</u>			
VLDL	40.1	9.8	50.1
LDL	20.5	11.6	67.9
<u>Lp-B:E</u>			
VLDL	ND	ND	ND
LDL	9.0	17.9	72.9

(Alaupovic, Prog.Lipid Res. 1991;30:105-138)

Percent Apolipoprotein Composition of Simple ApoB-containing Lipoprotein Families VLDL and LDL

Density Class	Apolipoprotein B	Apolipoprotein E
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%

Lp-B

VLDL	100	--
LDL	100	--

Lp-B:E

VLDL	ND	ND
LDL	75	25

(Alaupovic, Prog.Lipid Res. 1991;30:105-138)

Percent Lipid Composition of Complex ApoB-containing Lipoprotein Families VLDL and LDL

Density Class	Triglycerides	Cholesterol	Cholesterol Esters
		%	
<u>LP-B:C</u>			
VLDL	58.0	10.9	31.1
LDL	40.7	16.2	43.1
<u>Lp-B:C:E</u>			
VLDL	60.0	13.5	26.5
LDL	48.7	16.5	34.7
<u>LpA-II:B_{complex}</u>			
VLDL	69.5	11.0	19.2
LDL	33.6	15.7	50.5

(Alaupovic, Prog.Lipid Res. 1991;30:105-138)

**Percent Apolipoprotein Composition of Complex
ApoB-Containing Lipoprotein Families
VLDL and LDL**

Density Classes	Apolipoproteins						
	A-II	B	C-I	C-II	C-III	D	E
	%						
<u>LP-B:C</u>							
VLDL	–	53.8	8.9	6.4	30.5	–	–
LDL	–	69.7	6.5	3.1	20.6	–	–
<u>LP-B:C:E</u>							
VLDL	–	45.8	6.2	5.8	19.1	–	22.7
LDL	–	75.4	4.7	1.5	6.3	–	11.7
<u>LP-A-II:Bcomplex</u>							
VLDL	4.6	45.0	7.8	6.3	20.3	0.9	14.4
LDL	5.4	69.0	ND	1.0	6.7	5.9	11.9

ND = not determined

Apolipoproteins were measured by electroimmunoassays

(Alaupovic, Prog.Lipid Res. 1991;30:105-138)

Metabolic Properties of ApoA-containing Lipoproteins

Metabolic Property	LpA-I	LpA-I:A-II	Authors
Binding to HepG2 cells at 4 ° C	Higher	Lower	Kilsdonk et al.
Promotion of cholesterol efflux from adipose tissue cells	Agonist	Antagonist	Barbaras et al.
Association with LCAT and CETP activities	Carrier	Poor carrier	Cheung et al.
In vitro kinetics	Faster catabolic rate	Slower catabolic rate	Brewer et al.
Acceptor or ApoC and ApoE released during lipolysis of TG-rich lipoproteins	Secondary acceptor	Primary acceptor	Alaupovic et al. James et al.

(Reviewed in Alaupovic, Methods in Enzymology. Plasma Lipoproteins, Part C, Quantitation. San Diego, Academic Press, Inc. pp 32-60)

Differences in the Metabolic Properties of ApoB-containing Lipoprotein Families

Metabolic Property	Measurement	Density Class	Lipoprotein Particles
Substrate Reactivity for Lipoprotein Lipase	K_1 -values	VLDL	Lp-B:C:E > Lp-B:C > Lp-A-II:B:C:D:E
Binding to HeLa Cells	K_d -values	VLDL LDL	Lp-B:E > Lp-B > Lp-B:C:E > Lp-B:C
Binding to HepG2 Cells	K_d -values	LDL	Lp-B:E > Lp-B
Inhibition of Intracellular Sterol Synthesis	% Inhibition	LDL	Lp-B:C < Lp-B
Accumulation of Neutral Lipids and ApoB In THP1 Macrophages	Fluorescence Intensity	WP	Lp-B:C:E > Lp-B:C > Lp-A-II:B:C:D:E > Lp-B

(Reviewed in Alaupovic, Methods in Enzymology. Plasma Lipoproteins, Part C, Quantitation. San Diego, Academic Press, Inc. pp 32-60)

Concentrations of LpA-I and LpA-II Particles in Normolipidemic Patients with Angiographically Documented Coronary Artery Disease (CAD)

Subjects	TC	HDL-C	ApoA-I	LpA-I	LpA-I:A-II
With CAD	178 ± 49	44 ± 13	105 ± 19	30 ± 17	75 ± 17
Without CAD	190 ± 42	55 ± 13**	112 ± 20	41 ± 17*	72 ± 13
Controls	191 ± 43	59 ± 27**	131 ± 28**	52 ± 22**	79 ± 14

* p < 0.005

** p < 0.001

(Puchois et al. Atherosclerosis 68:35, 1987)

Monitored Atherosclerosis Regression Study (MARS)

Purpose

A prospective, randomized, placebo-controlled coronary angiographic trial, the purpose of which was to determine the effect of prolonged (2 years) lovastatin (80 mg/day) therapy on coronary angiographic findings (progression/non-progression) in subjects with moderate hypercholesterolemia and coronary artery disease. Coronary status was assessed by global change score and quantitative coronary angiography.

On-Trial Levels of Lipids, Apolipoproteins and Lipoprotein Families in MARS Subjects Classified by GCS as Progressors and Non-Progressors

	Lovastatin Treated			Placebo Treated		
	Non-Progressors (69%)	Progressors (31%)	p	Non-Progressors (42%)	Progressors (58%)	p
	mg/dL			mg/dL		
Triglycerides	121 ± 44	151 ± 77	0.32	136 ± 61	229 ± 121	0.03
ApoC-III-V+L	4.4 ± 2.2	4.9 ± 1.8	0.32	4.8 ± 2.0	6.9 ± 2.9	0.05
Lp-B	68 ± 13	66 ± 16	0.87	111 ± 17	114 ± 23	0.82
Lp-B _c	7.9 ± 5.2	15.1 ± 8.5	0.02	4.9 ± 3.1	7.3 ± 4.6	0.20
Lp-A-II:B:C:D:E	5.7 ± 4.5	8.5 ± 4.1	0.02	7.3 ± 3.1	9.2 ± 4.5	0.25

Mean ± SD

(Alaupovic, et al., Arterioscler.Thromb.Vasc.Biol. 1997;17:715-722)

**Relative Risk (95% CI) for Coronary Artery Lesion Progression
Based on Concentrations of Triglyceride-rich Lipoproteins
(Lp-Bc Tertiles) in a Subpopulations of MARS Subjects Classified by
Global Change Score as Non-Progressors or Progressors**

Lp-B_c (Tertiles)	Non-Progression	Progression	RR (95% CI)	p-trend
<u>Combined Lovastatin- and Placebo-Treated Groups *</u>				
≤ 6.0	14 (64%)	8 (36%)	1.0	0.008
> 6.0 – 9.9	14 (70%)	6 (30%)	0.8 (0.2 – 3.2)	
>9.9	7 (33%)	14 (67%)	4.4 (1.1 – 16.9)	

Lp-B_c = LpB:C + Lp-B:C:E expressed in mg/dL of apoB

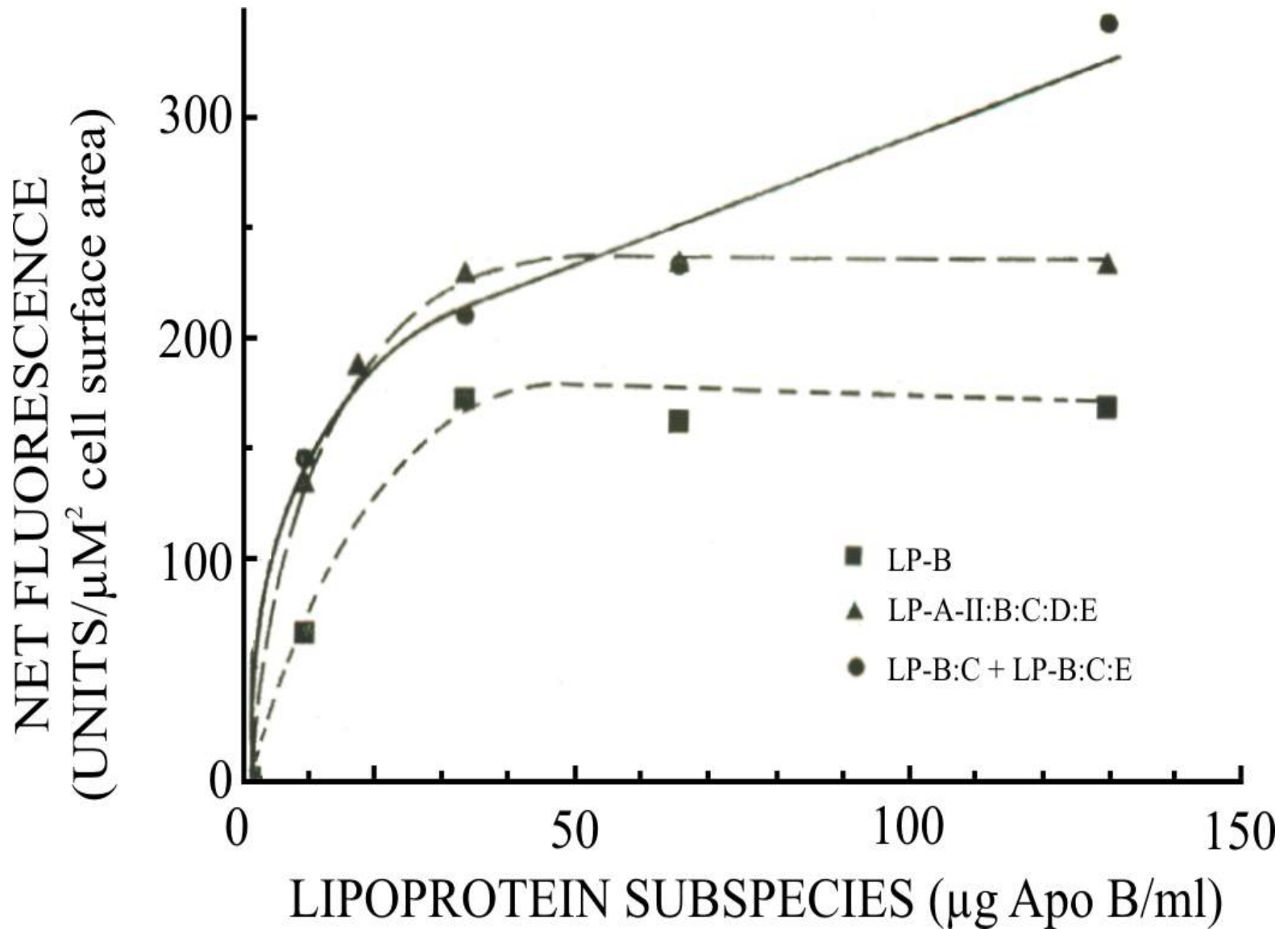
*** Adjusted for Treatment Group**

(Alaupovic, et al., Arterioscler.Thromb.Vasc.Biol. 1997;17:715-722)

Monitored Atherosclerosis Regression Study (MARS)

Conclusions

1. Results of MARS trial have demonstrated that after lowering LDL-cholesterol below 90 mg/dL or Lp-B particles below 70 mg/dL, increased concentrations of intact or partially delipidized triglyceride-rich Lp-B:C + Lp-B:C:E and Lp-A-II:B:C:D:E particles become the most significant risk factors for atherosclerotic lesion progression.
2. The increased levels of apoC-III bound to apoB-containing lipoproteins (VLDL + LDL) have emerged as a very useful predictor of coronary artery disease progression.
3. Direct measurement of individual apoB-containing lipoproteins has shown that intact or partially delipidized triglyceride-rich lipoproteins (Lp-B:C, Lp-B:C:E and LpA-II:B:C:D:E) have atherogenic capacities similar to, if not greater, than that of cholesterol-rich Lp-B particles.



(Koren et al., Atherosclerosis 1994;109:217-218)

Concentrations of ApoB-containing Lipoproteins in VLDL of Type II Diabetics With and Without Vascular Disease

Patients	Lp-B	Lp-B:C	Lp-B:C:E	<u>Lp-B:C</u> Lp-B:C:E
	mg/dL			
Vascular Disease (N = 8)	1.4	15.4	9.8	<u>1.56</u>
No Vascular Disease (N = 4)	3.1	3.2	7.6	0.42

(Alaupovic et al., Diabetes 1992;41(Suppl.2):18-25)

Type II Diabetes

Purpose: The purpose of this cross-sectional study was to determine the relation between macroangiopathy and especially coronary heart disease and lipid variables in 188 patients with type II diabetes mellitus

(N. Gervaise et al., Diabetologia 43:703-708, 2000)

Type II Diabetes

Variables Independently Associated with Macroangiopathy in Logistic Regression Models

Variables (reference)	Odds Ratio (CI 95%)	p value
LpB:C (< 17 mg mg/L)	2.73 (1.33 - 5.6)	0.005
Microalbuminuria (< 30 mg/24 h)	2.45 (1.2 - 4.9)	0.01
Gender (Female)	2.38 (1.2 - 4.7)	0.01
Age (< 60 years)	2.28 (1.1 - 4.7)	0.02
Duration of diabetes (< 10 years)	2.11 (1.04 - 4.3)	0.03

Models included age, duration of diabetes, BMI, HbA_{1c}, gender, drugs, microalbuminuria, hypertension, apoC-III, LpB:C and TG

Type II Diabetes

Relation Between LpB:C and Macroangiopathy or CHD

	Quartiles				Trend
	1	2	3	4	
LpB:C (mg/L)	< 10	≥ 10 to < 16	≥ 16 to < 23	≥ 23	
Macroangiopathy Odds Ratio	1	2.06	3.46	6.24	p < 0.004
CHD Odds Ratio	1	2.71	4.98	4.45	p < 0.006

ApoB Lipoproteins Containing ApoC-III as Risk Factors for Recurrent Coronary Events in Diabetic Patients Ancillary CARE Study

Purpose: To determine in a prospective nested case-control study the relation between apoB lipoproteins containing apoC-III and recurrent cardiovascular events in patients with type II diabetes mellitus.

Patients: Among 242 diabetic patients with myocardial infarction from the CARE trial, 121 patients with a recurrent cardiovascular event were compared with 121 patients without an event during 5 years follow-up.

(S-J Lee et al. *ATVB* 23:853-858, 2003)

Concentrations of ApoB-containing Lipoproteins of Type II Diabetics With and Without Recurrent Coronary Events

Variables	Controls (n = 121)	Cases (n = 121)	P-value
mg/dL			
<u>VLDL</u>			
Lp-B:C:E	1.9 ± 1.2	1.8 ± 1.5	0.9
Lp-B:C	0.89 ± 0.80	0.85 ± 0.84	0.9
Lp-B	3.0 ± 1.6	3.5 ± 2.2	0.0009
<u>IDL + LDL</u>			
Lp-B:C:E	4.7 ± 1.7	5.4 ± 2.4	0.0001
Lp-B:C	4.6 ± 2.4	5.6 ± 3.2	0.0002
Lp-B	83 ± 25	86 ± 27	0.003

Mean ± SD Concentrations of apoB-containing lipoproteins are expressed in terms of apoB.

(S-J Lee et al. ATVB 23:853-858, 2003)

Relative Risks for Lp-B and Lp-B:C (LDL + IDL) in Type II Diabetics with Recurrent Coronary Events

		Quartiles			
		1	2	3	4
<u>Lp-B:C</u> Adjusted	Mean	4.5	5.9	7.2	10.4
	RR (CI)	1	3.0 (1.2 – 7.6)	1.7 (0.6 – 4.7)	<u>6.6 (2.6 – 17)</u>
	P-value		0.02	0.3	< 0.0001
<u>Lp-B</u> Adjusted	Mean	42	55	65	86
	RR (CI)	1	1.0 (0.4 – 2.2)	1.6 (0.7 – 3.7)	<u>2.2 (0.9 – 5.0)</u>
	P-value		0.9	0.2	0.07

Mean values are apoB concentrations (mg/dL)

Adjusted for covariates : TG, LDL-C, HDL-C, age, sex, exercise, waist circumference, CABG, angina, glucose and hypoglycemic use

(S-J Lee et al. ATVB 23:853-858,2003)

Lipoprotein A-I and Lipoprotein A-I:A-II in Normolipidemic and Dyslipoproteinemic Subjects

Dyslipoproteinemias	HDL-C	ApoA-I	Lp-A-I	Lp-A-I:A-II
mg/dL				
Moderate Hypercholesterolemia (n = 253)	38 ± 10 ^a	127 ± 20 ^a	31.4 ± 4.8 ^a	94.5 ± 18.4 ^a
Hypertriglyceridemia (n = 16)	36 ± 9 ^a	123 ± 18 ^b	33 ± 6	97 ± 17
Chronic Renal Failure (n = 93)	38.7 ± 15 ^a	114 ± 22 ^a	34 ± 8	80 ± 21 ^a
Diabetes, Type II (n = 224)	44 ± 9 ^a	114 ± 24 ^a	30 ± 6 ^a	84 ± 19 ^a
Normolipidemia (n = 238)	51 ± 14	139 ± 27	34 ± 7.5	104 ± 22

Mean ± SD: Normolipidemic vs Dyslipoproteinemic Subjects:

a = p < 0.0001

b = p < 0.05

Effect of Drugs on ApoA-containing Lipoprotein Particles in Subjects with Combined Hyperlipoproteinemia

Drugs	Lipoprotein A-I		Lipoprotein A-I:A-II		Authors
	Base	% Change	Base	% Change	
	(mg/dL)	(%)	(mg/dL)	(%)	
Cholestyramine	45	<u>+15</u>	93	<u>+41</u>	Fruchart et al.
Simvastatin	44	+6	86	+2	Fruchart et al.
Pravastatin	43	+5	96	<u>+33</u>	Fruchart et al.
Lovastatin	26	+4	88	+8	Alaupovic et al.
Fluvastatin	45	-2	81	+4	Banga et al.
Fenofibrate	44	-15	78	+22	Fruchart et al.
Gemfibrozil	39	-2	76	-1	Betteridge et al.
Niacin	67	<u>+22</u>	86	-15	Atmeh et al.

Duration of treatment was 12 weeks except for lovastatin (2 years), fluvastatin (1 year) and niacin (2 weeks)

Apolipoprotein B-containing Lipoprotein Families in Normolipidemic and Dyslipoproteinemic Subjects

Dyslipoproteinemias	ApoB	Lp-B	Lp-B:C	Lp-B:E + Lp-B:C:E	Lp-A-II:B:C:D:E
mg/dL					
Phenotype IIA (n = 35)	142 ± 15 ^a	73 ± 7.7 ^a	16.4 ± 4 ^a	18.3 ± 4.7 ^a	20.8 ± 5.9 ^a
Phenotype IIB (n = 26)	171 ± 15 ^a	91 ± 10.7 ^a	21.7 ± 7.1 ^a	21.7 ± 8.2 ^a	31.5 ± 10.7 ^a
Chronic Renal Failure Before Dialysis (n = 15)	137 ± 40 ^a	82 ± 30 ^a	20.3 ± 10 ^a	22.3 ± 7.5 ^a	12.0 ± 5.6
Diabetes, Type II (n = 7)	119 ± 17 ^b	69 ± 18 ^d	14.0 ± 7.2	21.0 ± 18.0 ^c	15.0 ± 5.3
Normolipidemia (n = 75)	93 ± 19	56 ± 12	10.6 ± 5.3	12 ± 5.3	13.4 ± 8.0

Mean ± SD: Normolipidemic vs Dyslipoproteinemic Subjects a = p < 0.0001, b = p < 0.001
 c = p < 0.005, d = p < 0.01

Effects of Drugs on ApoB-containing Lipoprotein Families in Subjects with Dyslipoproteinemias

Drugs	Lipid Disorder	Lp-B		Lp-B:C		Lp-A-II:B:C:D:E	
		Base (mg/dL)	%Change (%)	Base (mg/dL)	%Change (%)	Base (mg/dL)	%Change (%)
Niacin + Colestipol	MHC	100	-49	5.1	+10	NA	
Niacin + Simvastatin	MHC	65	-14	13.2	-31	18.6	-34
Simvastatin	CH	69	-18	11.0	-10	22.0	-46
Atorvastatin	CH	84	-23	15.0	-26	27.0	-29
Fluvastatin	CRF	82	-21	20.0	-14	11.0	-2
Atorvastatin	CRF	90	-38	17.0	-32	20.0	-6
Gemfibrozil	Phenotype V	39	+37	32.0	-27	NA	
Fenofibrate	CH	117	-17	25.0	-33	9.0	+40

MHC = Moderate hypercholesterolemia
CRF = Chronic renal failure

CH = Combined hyperlipoproteinemia
NA = Not available

Conclusions

1. Lipoprotein families defined by apolipoprotein composition are the fundamental chemical and metabolic entities of plasma lipoprotein system.
2. The apoA-containing lipoproteins consist of three major and apoB-containing lipoproteins of five major lipoprotein families.
3. Each lipoprotein family is a polydisperse system of particles homogeneous with respect to qualitative apolipoprotein composition.
4. Chemically distinct lipoprotein families have distinct metabolic properties

Conclusions (Cont.)

5. Dyslipoproteinemias are characterized, in general, by quantitative rather than qualitative differences in the concentrations of apoA- and apoB-containing lipoprotein families.
6. ApoA-containing lipoproteins seem to differ in their antiatherogenic capacities and apoB-containing lipoproteins in their atherogenic potentials. The available evidence suggests that LpB:C particles may have a greater atherogenic potential than LpB particles.
7. The levels of lipoprotein families seem to be selectively affected by pharmacologic and dietary interventions.
8. Determination of individual apoA- and apoB-containing lipoprotein families and the distribution of apoC-III bound to these two lipoprotein classes may represent useful biomarkers for identifying subjects with increased risk of atherosclerotic disease and for selecting specific treatments targeted at reducing the undesirable and/or elevating the desirable or protective lipoprotein families.