

LipoSEARCH

Lipoprotein Profiling Service



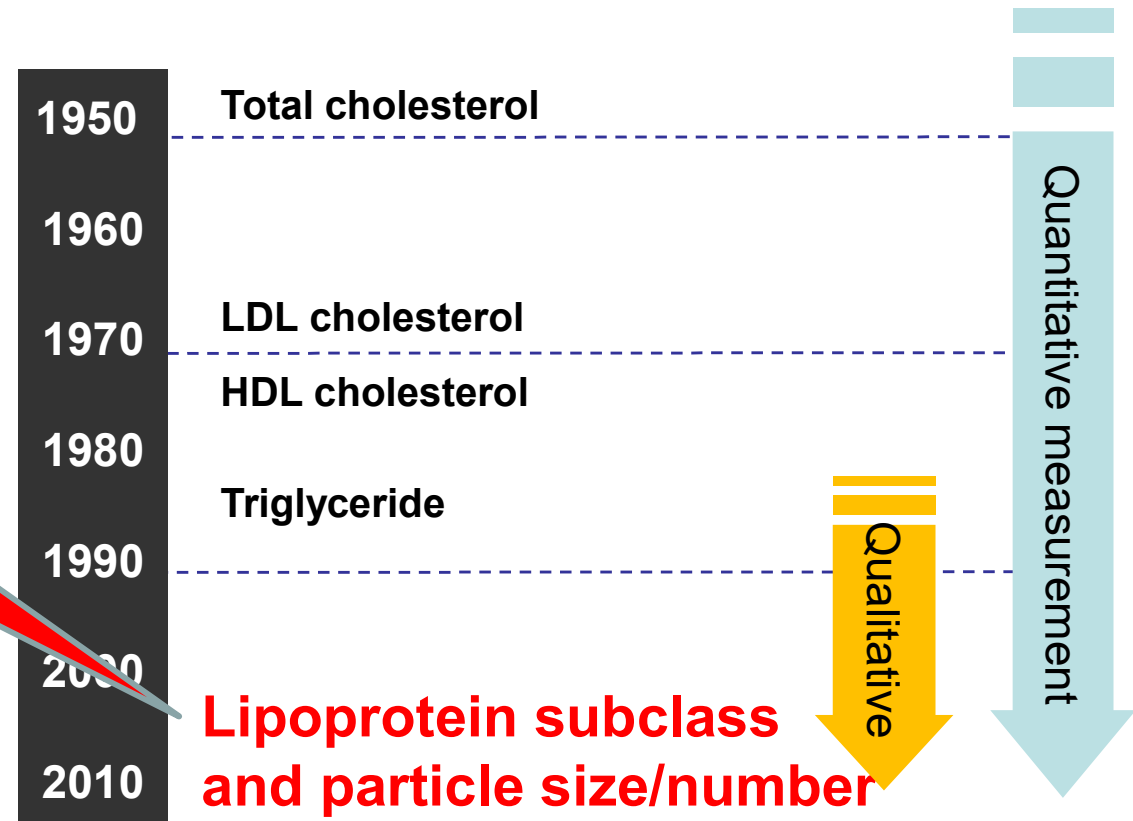
IBL Group company

Detailed and accurate analysis of Lipoprotein profiling become significantly important for exploring what is the real risks of cardiovascular diseases.

Need to carefully look into bad Cholesterol in LDL subclass

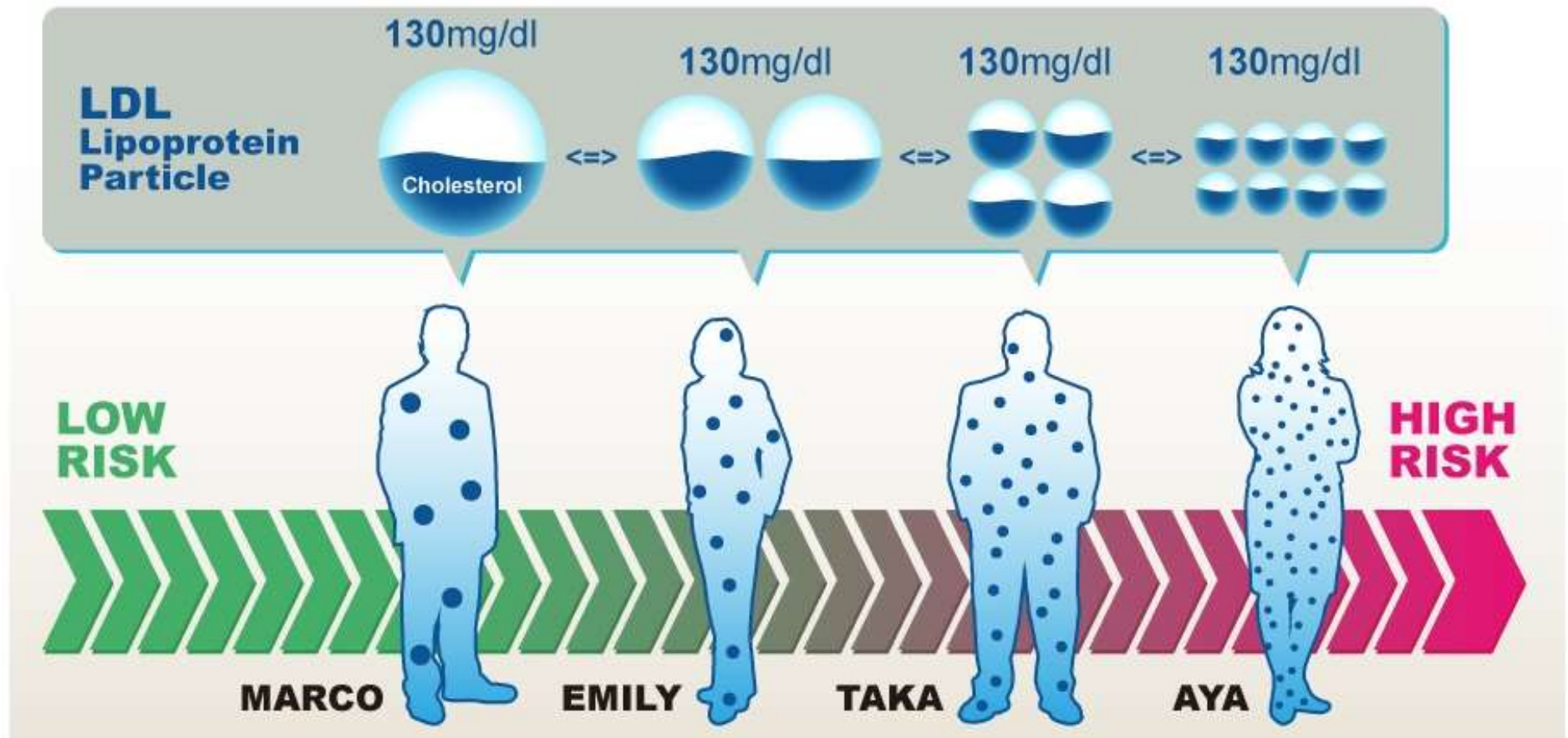


LipoSEARCH is the best profiling system in the world.



New Insight of Lipoprotein Particle Number

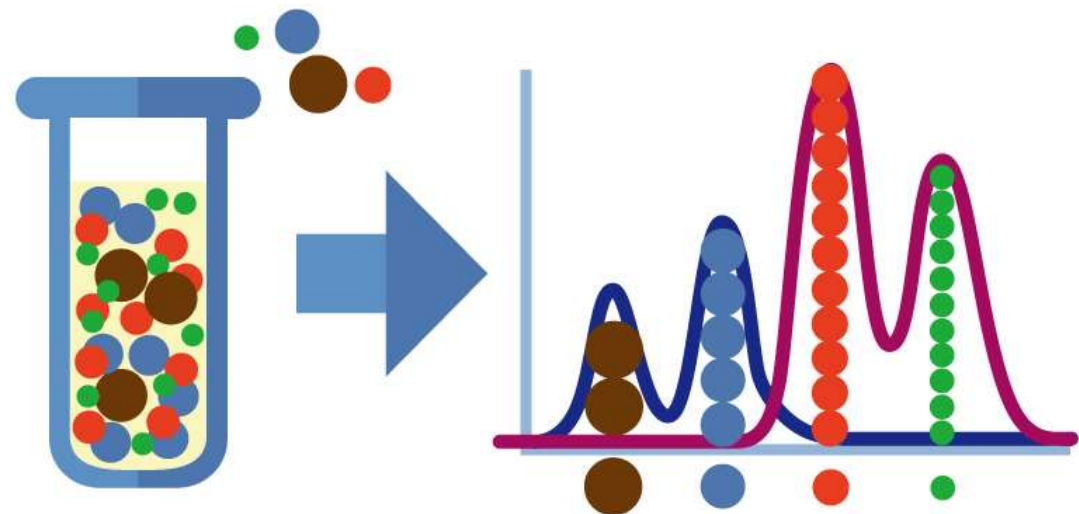
The theory of analysis of lipoprotein particle number based on spherical particle model by LipoSEARCH has been published by our senior technical advisor, Dr. Okazaki, professor emeritus at Tokyo Medical and Dental University.
(*J.Oleo Sci.* 65, (4) 265-282, 2016)



What is LipoSEARCH?

LipoSEARCH is an **Advanced Lipoprotein Testing Service**

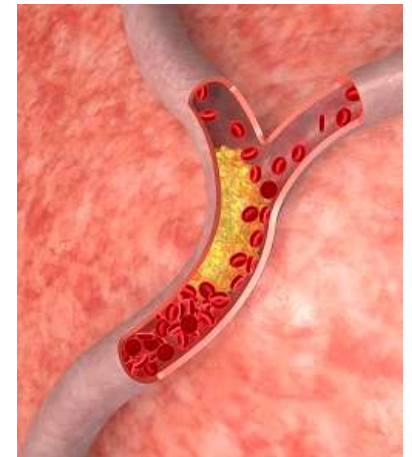
- **Most accurate whole lipoprotein profiles by GP-HPLC with patented data analysis algorithm**
 - High reproducibility
- **Required only low double digit μ L of blood sample**
 - Human: 45 μ L; other speices: 35 μ L
- **Profile any kind of species**
 - human, monkey, mice, rat, rabbit, hamster, canaine, fish, etc.



How LipoSEARCH can be used?

LipoSEARCH has been utilized:

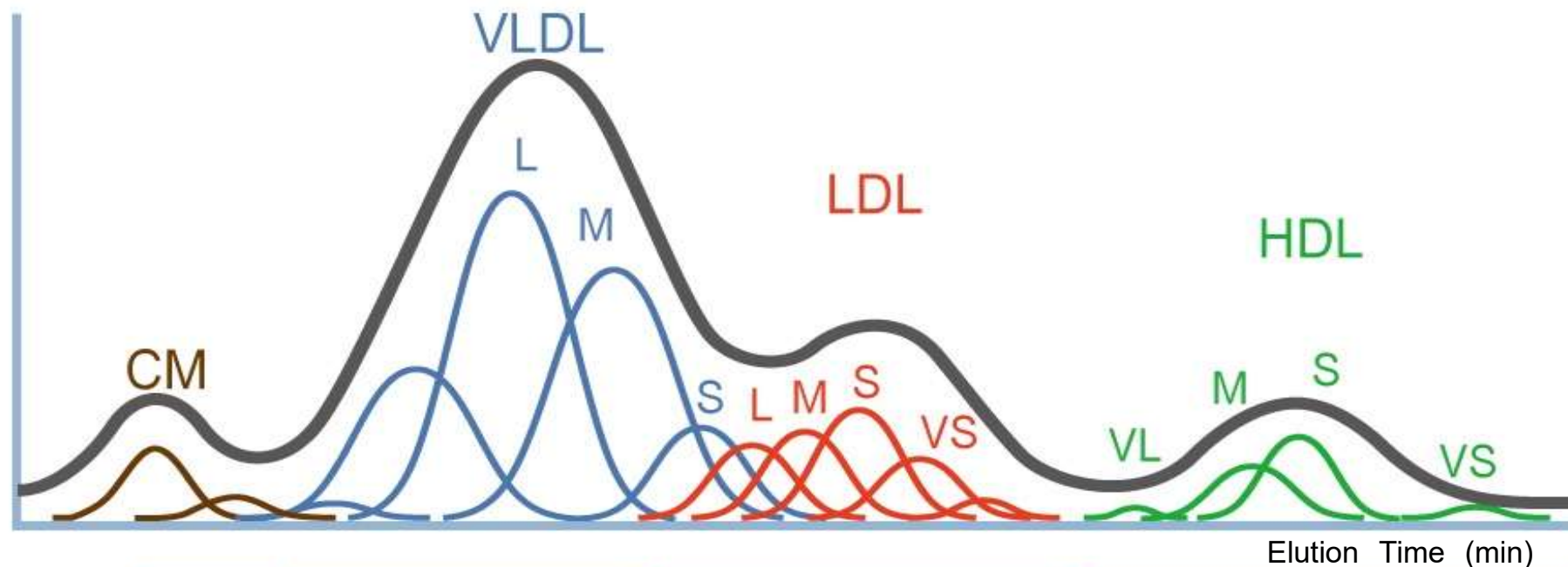
- **over 550 publications**
- **for drug discovery and R&D**
 - **atherosclerosis, dyslipidemia, cardio vascular diseases, cerebral stroke, diabetes, NASH, NAFLD, depression and Alzheimer's diseases, etc.**
- **over a number of clinical studies and several phase II and III clinical trials**
- **for veterinary practice**
- **to prove efficacy for functional food**



LipoSEARCH provides detailed lipoprotein profiling data.

- ✓ Total cholesterol & triglyceride level
- ✓ Cholesterol and triglyceride in **4 major classes** (CM, VLDL, LDL, HDL)
- ✓ Cholesterol and triglyceride in **20 sub-classes** defined by a particle size
 - Including quantitative determination of **small, dense LDL:**
a high risk marker of atherosclerosis
- ✓ **Particle size**
- ✓ **Particle number**
- ✓ Option: free phospholipid, cholesterol, and glycerol level

20 subclasses of Lipoproteins



Major Class	CM		VLDL					LDL						HDL						
Component Peak No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Subclass Name	CM1	CM2	VLDL 1	VLDL 2	VLDL 3	VLDL 4	VLDL 5	LDL 1	LDL 2	LDL 3	LDL 4	LDL 5	LDL 6	HDL 1	HDL 2	HDL 3	HDL 4	HDL 5	HDL 6	HDL 7
Major Subclass Name	CM		L VLDL			M VLDL		S VLDL	L LDL	M LDL	S LDL	VS LDL		VL HDL	L HDL	M HDL	S HDL	VS HDL		
Particle Diameter (nm)	>90	75	64	53.6	44.5	36.8	31.3	28.6	25.5	23	20.7	18.6	16.7	15	13.5	12.1	10.9	9.8	8.8	7.6

VL: Very Large, **L:** Large, **M:** Medium, **S:** Small, **VS:** Very Small

Example of Output Data

Total Cho & TG

Unit : mg/dl

	Total
Cho	237.93
TG	84.13

Major 4 fractions numeric data

Class	CM (>80nm)	VLDL (30-80nm)	LDL (16-30nm)	HDL (8-16nm)
Cho	0.05	21.36	138.21	78.31
TG	0.33	46.47	24.05	13.28

Detailed 20 fractions numeric data

Peak No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Particle Diameter (nm)	>90	75	64	53.6	44.5	36.8	31.3	28.6	25.5	23	20.7	18.6	16.7	15	13.5	12.1	10.9	9.8	8.8	7.6
Sub-Class			large VLDL			medium VLDL	small VLDL	large LDL	medium LDL	small LDL	very small LDL			very large HDL		large HDL	medium HDL	small HDL	very small HDL	
Cho	0.05	0.00	0.00	0.41	2.45	6.61	11.89	38.11	52.94	31.36	10.54	4.00	1.27	1.66	2.56	19.77	26.57	16.59	6.95	4.20
TG	0.18	0.15	0.46	5.30	15.48	15.88	9.35	9.70	7.98	4.02	1.50	0.61	0.23	0.31	0.23	3.10	4.61	2.67	1.04	1.32

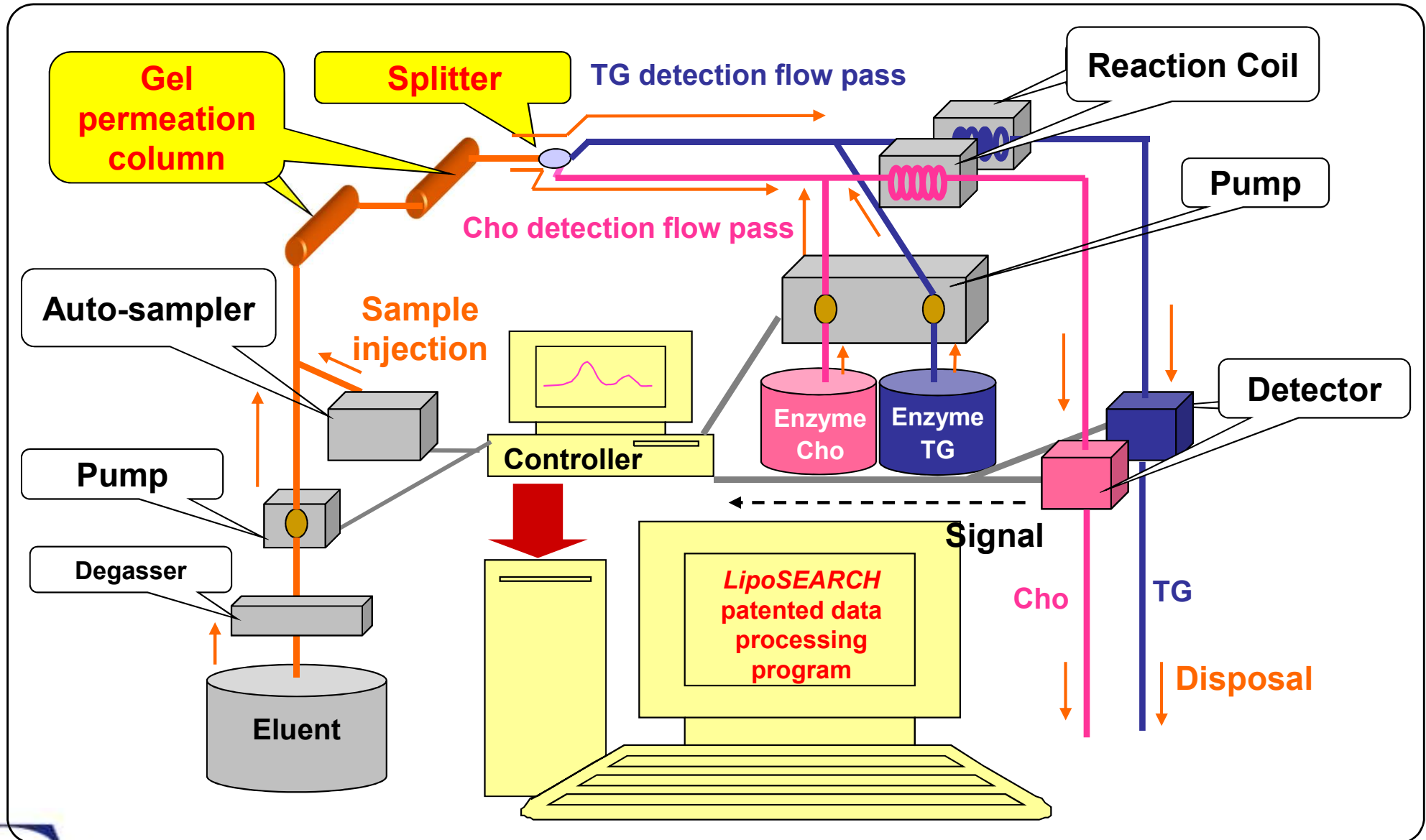
small, dense LDL-Cho

Comparison of LipoSEARCH with Other Methods

Method	LipoSEARCH (HPLC)	Ultracentrifugation	1D Electrophoresis (GGE)	Direct Method	NMR
Separation Mode	Particle Size	Specific Gravity	Electric Charge	Enzymic Reaction	Magnetic Resonance
Sample	0.01ml	0.7ml	0.05ml	1ml	0.6ml
Testing Time	25 min.	Over 270 min.	30 min.	10 min.	1min.
Major Classes	✓	✓	✓	△	✓
CM	✓	✓	×	×	?
VLDL	✓	✓	△	×	?
LDL	✓	✓	△	△	✓
HDL	✓	✓	△	✓	✓
Subclasses	✓	△	△	×	△
VLDL subclass	✓	△	?	×	✓
LDL subclass	✓	△	△	△	✓
HDL subclass	✓	△	△	×	✓
Particle Size	✓	×	△	×	✓
Particle Count	✓	×	×	×	✓
Fractionation	✓	✓	×	×	×
Reproducibility	✓	×	×	✓	△
Quantitativeness	✓	×	×	✓	△
Cost	△	×	△	✓	△

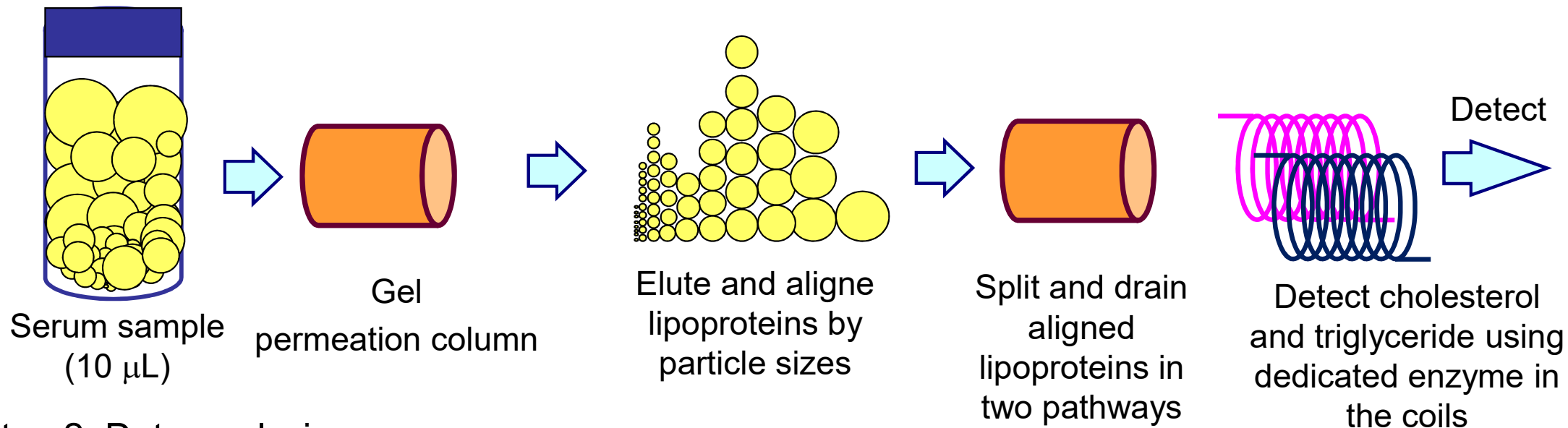
Mechanism of the HPLC-based Profiling System

Mechanism of the HPLC-based profiling system

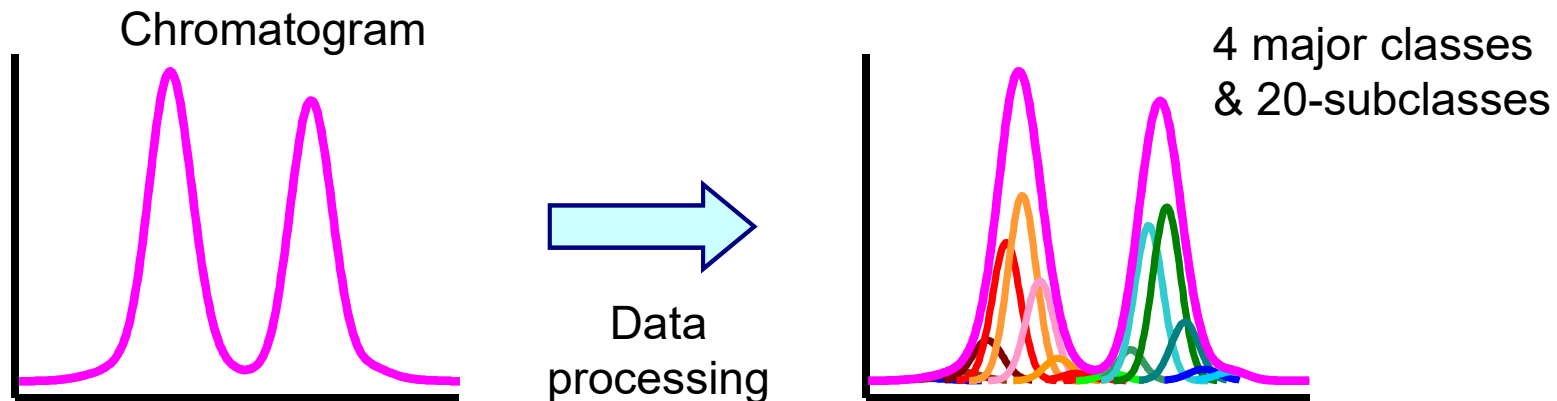


Mechanism of the HPLC-based Profiling System

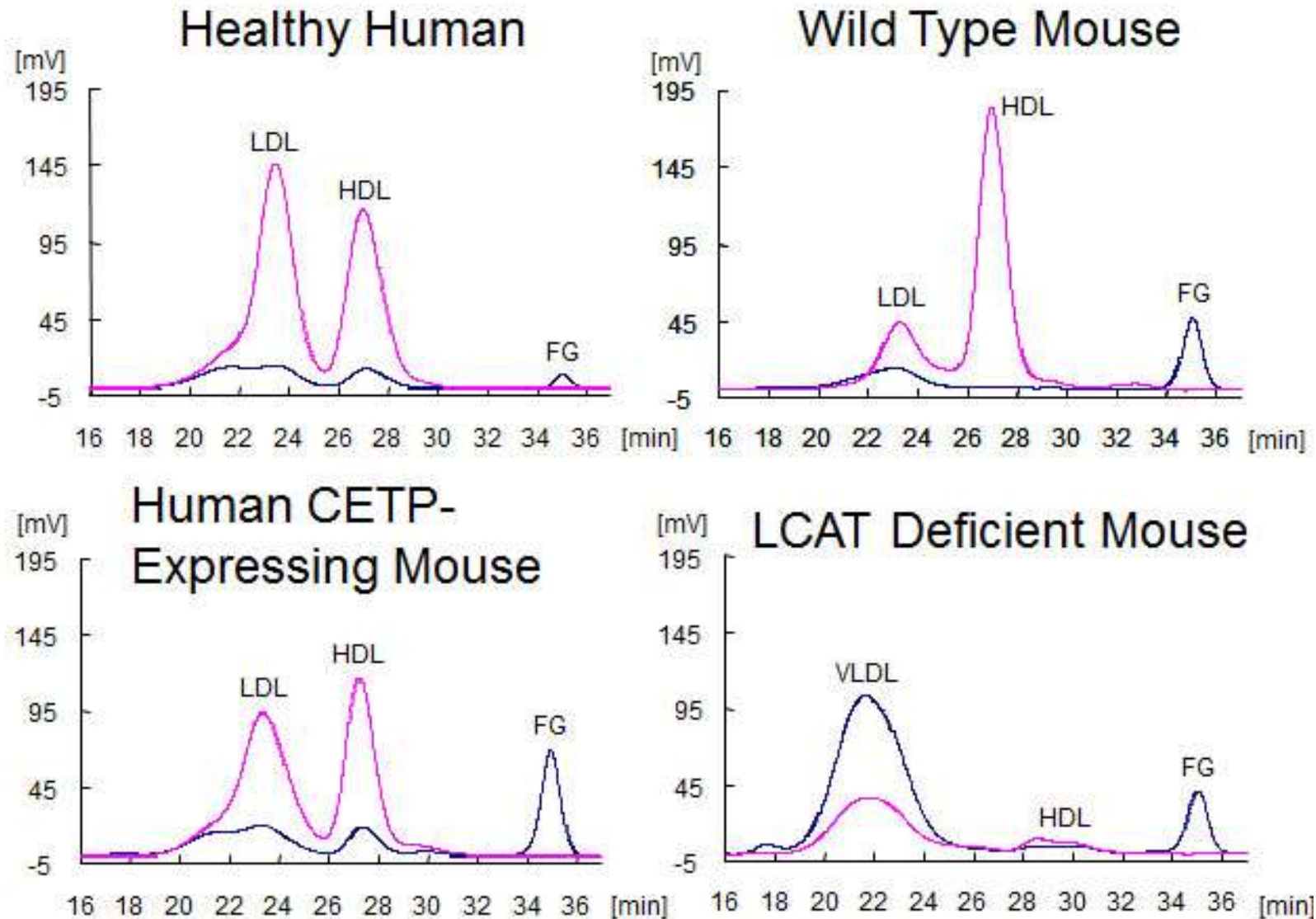
Step 1: Sample separation and enzymatic reaction



Step 2: Data analysis



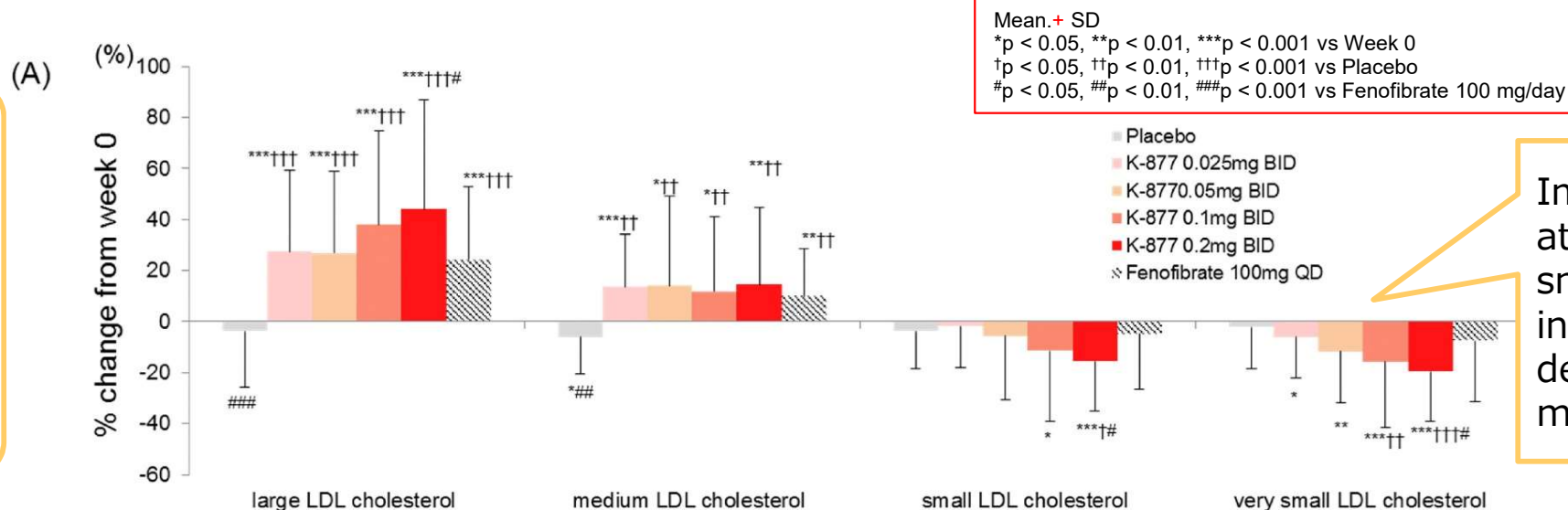
Example Data: Human and Murine Profiles



Wu C., Tsujita M., Okumura-Noji K., Usui S., Kakuuchi H., Okazaki M., Yokoyama S.:
Cholesterol ester transfer protein expressed in lecithin cholesterol acyltransferase-deficient mice. *Arterioscler Thromb Vasc Biol.* 2002; 22(8): 1347-1353.

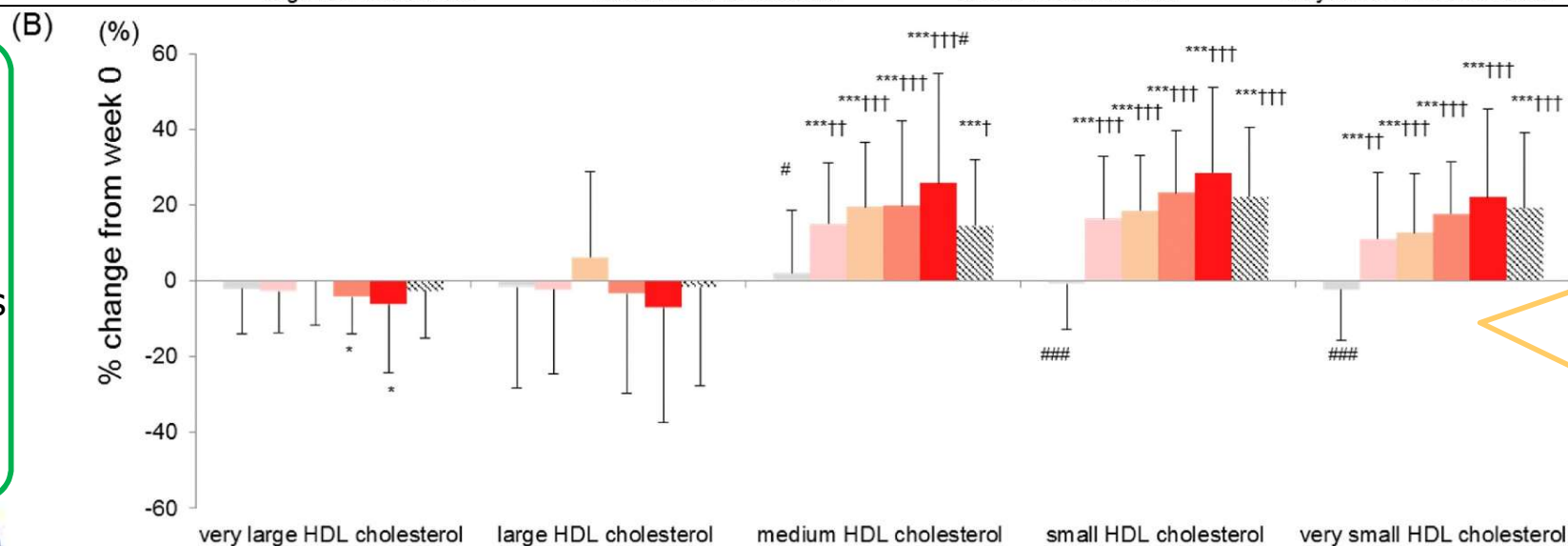
Data Example: Phase II Clinical Trial of Pemafibrate

LDL
subclass



Improvement of
atherogenic
small LDL-Cho.
in dose-
dependent
manner

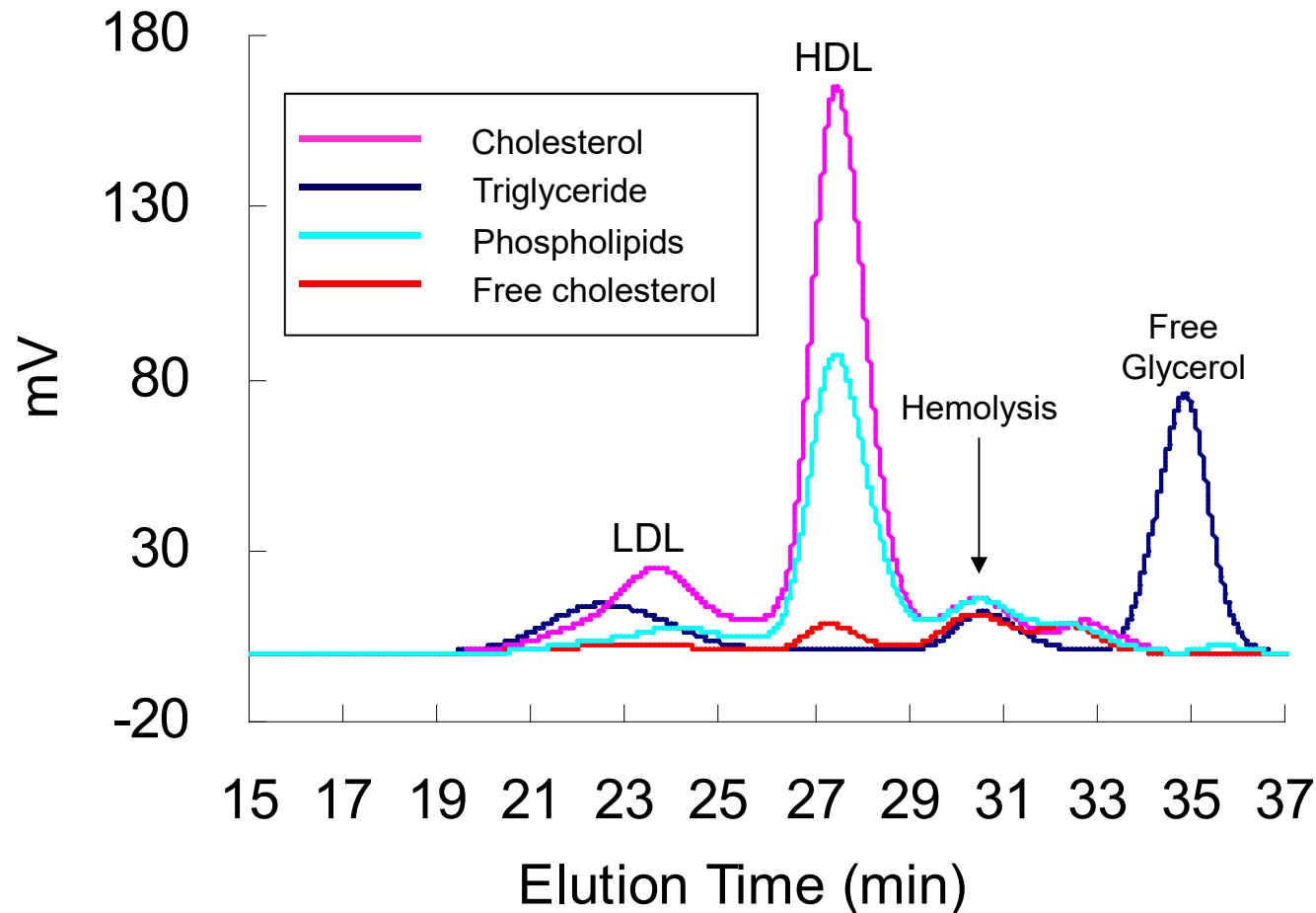
HDL
subclass



Improvement of
atherogenic
middle~small
HDL-Cho. in
dose-dependent
manner

Free Cholesterol and Phospholipids (Option Services)

Free Cholesterol and Phospholipids are also measurable with additional charge.



Preparation of Sample Shipment

- **Sample volume required**

- **Serum: Human 45 μ L or more , Animal 35 μ L or more (undiluted sample).**

Leave the sample at room temperature for 30 min after blood collection. Subsequently, after cooling the sample on ice, centrifuge at 3000 rpm for 15 min at 4 °C. Solidified chylomicrons are often observed in the top separating layer in samples obtained from the fat tolerance test. In such a case, dissolve these thoroughly using a pipette. Use of a blood collection tube with clot activator will facilitate pipetting because the layer of clot activator inserts between the serum and blood clot layer.

- **Plasma: Human 45 μ L or more , Animal 35 μ L or more (undiluted sample).**

After collection in a plasma separation tube with clotting factor inhibitor, the sample and the inhibitor are mixed by inversion so that they can react sufficiently. After cooling the sample on ice, the plasma is separated by centrifugation at 3000 rpm for 15 min at 4 °C. For samples obtained from the fat tolerance test, please follow the same procedure as that used with serum samples but use a plasma separation tube containing separating agents.

- **For samples of low concentration, such as culture supernatants and cerebrospinal fluid, please contact us before the shipping.**

N.B.

1. Filter sterilization is highly recommended in order to avoid denaturation of lipoprotein by bacteria-derived protease.
2. **In case that 45 μ L(human) or 35 μ L(animal) is not available, please enquire us in advance.**
3. Please enquire in advance if you require analysis of samples containing anticoagulant (* Heparin is NOT acceptable as an anticoagulant) or samples that may contain pathogens.
4. We recommend that samples be frozen immediately after the collection and stored at -80 °C until the shipment. Samples that are repeatedly frozen and thawed are not accepted.
5. Pooled samples should be avoided.

● Packing and shipping

- Supply the sample in a 0.5 to 2.0 mL microtube for centrifugation (e.g., Eppendorf tube) and firmly seal it with Parafilm.
- **Write the ID on each sample tube (e.g., sample name and/or number) using an indelible pen.**
- Pack sufficient cold insulators or dry ice along with your samples.
- If possible, ask the shipping company to keep your samples refrigerated.

N.B.

1. We are NOT responsible for the sample preparation, the arrangement of shipping, any mishandling by the shipping company during the transportation (e.g., spillage during the transportation), and any resultant loss.
2. We will NOT store and/or return your samples.
3. Samples that are repeatedly frozen and thawed are not accepted.

● Consignee/Shipping address

Analysis Center, Skylight Biotech Inc.
100-4, Sunada, Iijima
Akita-shi, Akita 011-0911, Japan
Tel: +81-18-880-5060

- ◆ If you use FedEx, we will separately arrange a local chilled transport in Japan. Please note that the above address should be designated as the final destination in your arrangement with FedEx.
- ◆ The shipping schedule should be arranged so that the samples can be delivered to the analysis center on weekdays or Saturday. We cannot receive them on Sunday or any national holiday in Japan.

Preparation of Sample Shipment (Example)

1



Place shock absorption between sample container and dry ice.

2



Place a cardboard and dry ice on the sample container.

3



Place an additional shock absorption on the top.

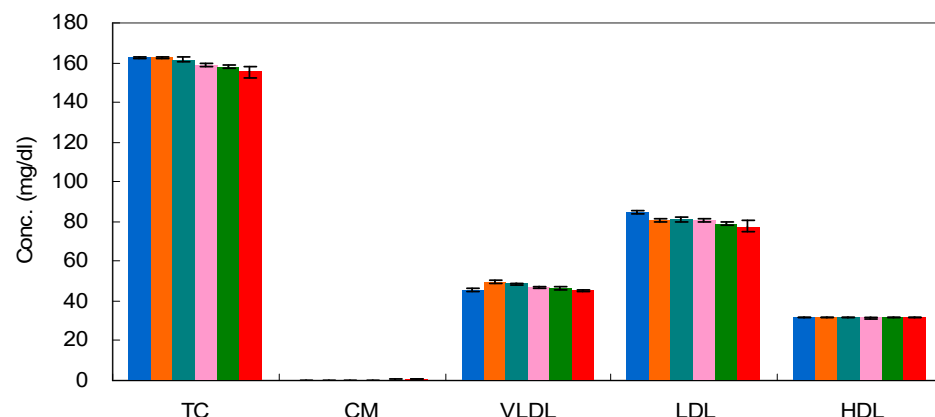
4



Close the box with the lid and seal it with a packing tape.

Effects of freezing and thawing on samples

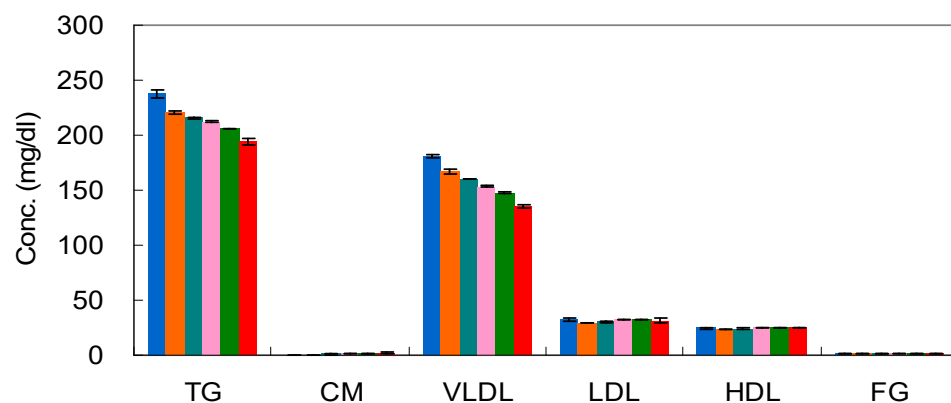
Effects of freezing and thawing on cholesterol level



* Human serum, average of three measurements

# of times	TC	s.d.	CM	s.d.	VLDL	s.d.	LDL	s.d.	HDL	s.d.
0	163	0.64	0.01	0.00	45.8	0.93	84.8	1.06	32.0	0.12
1	163	0.47	0.07	0.01	49.8	0.77	80.7	0.78	31.9	0.19
2	162	1.16	0.15	0.01	48.6	0.64	81.1	1.17	31.8	0.24
3	159	0.83	0.27	0.01	46.9	0.28	80.5	0.74	31.5	0.29
4	158	0.83	0.40	0.02	46.5	0.51	79.3	0.74	31.6	0.08
5	155	2.80	0.55	0.01	45.3	0.35	77.6	3.10	31.7	0.08

Effects of freezing and thawing on triglyceride level



* Human serum, average of three measurements

# of times	TG	s.d.	CM	s.d.	VLDL	s.d.	LDL	s.d.	HDL	s.d.
0	238	3.57	0	0.18	181	2.03	33	1.44	24	0.56
1	220	1.9	0.4	0.16	167.0	1.66	29.4	0.50	23.6	0.27
2	216	0.8	0.8	0.08	160.4	0.37	30.3	0.54	24.2	0.14
3	212	1.1	1.1	0.17	153.6	0.91	32.3	0.31	25.1	0.29
4	206	0.4	1.6	0.26	147.5	0.98	32.2	0.27	24.8	0.46
5	194	3.4	2.1	0.15	135.4	0.92	31.4	1.77	25.1	0.63

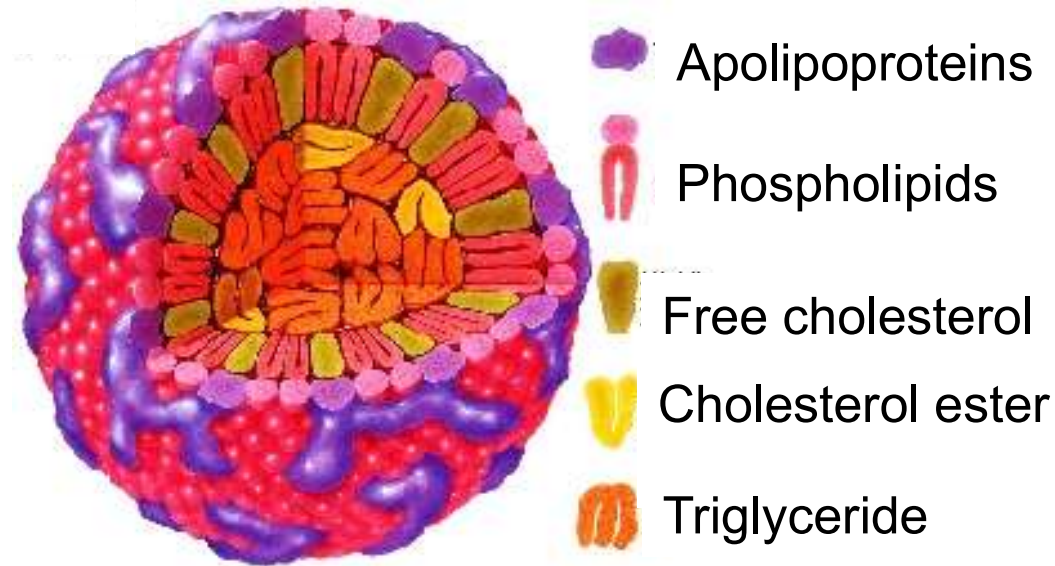
- Recent Advances in Analytical Methods on Lipoprotein Subclasses: Calculation of Particle Numbers from Lipid Levels by Gel Permeation HPLC Using “Spherical Particle Model” Okazaki M. and Yamashita S.
J.Oleo Sci. 65, (4) 265-282 (2016)
- LipoSEARCH®; Analytical GP-HPLC method for lipoprotein profiling and its applications. Toshima G., et al.
J.Biol.Macromol. 2013,13(2):21-32
- A new on-line dual enzymatic method for simultaneous quantification of cholesterol and triglycerides in lipoproteins by HPLC. Usui S., et al.
J Lipid Res. 2002, 43(5), 805-814.
- Identification of unique lipoprotein subclasses for visceral obesity by component analysis of cholesterol profile in HPLC. Okazaki M., et al.
Arterioscler Thromb Vasc Biol. 2005;25(3):578-584.

Publications using LipoSEARCH are found at <http://www.lipo-search.com/eng/literature/>

Appendix

What is Lipoprotein?

Lipoprotein



Cholesterol and triglyceride are NOT flowed in blood stream without a carrier because they are **hydrophobic**.



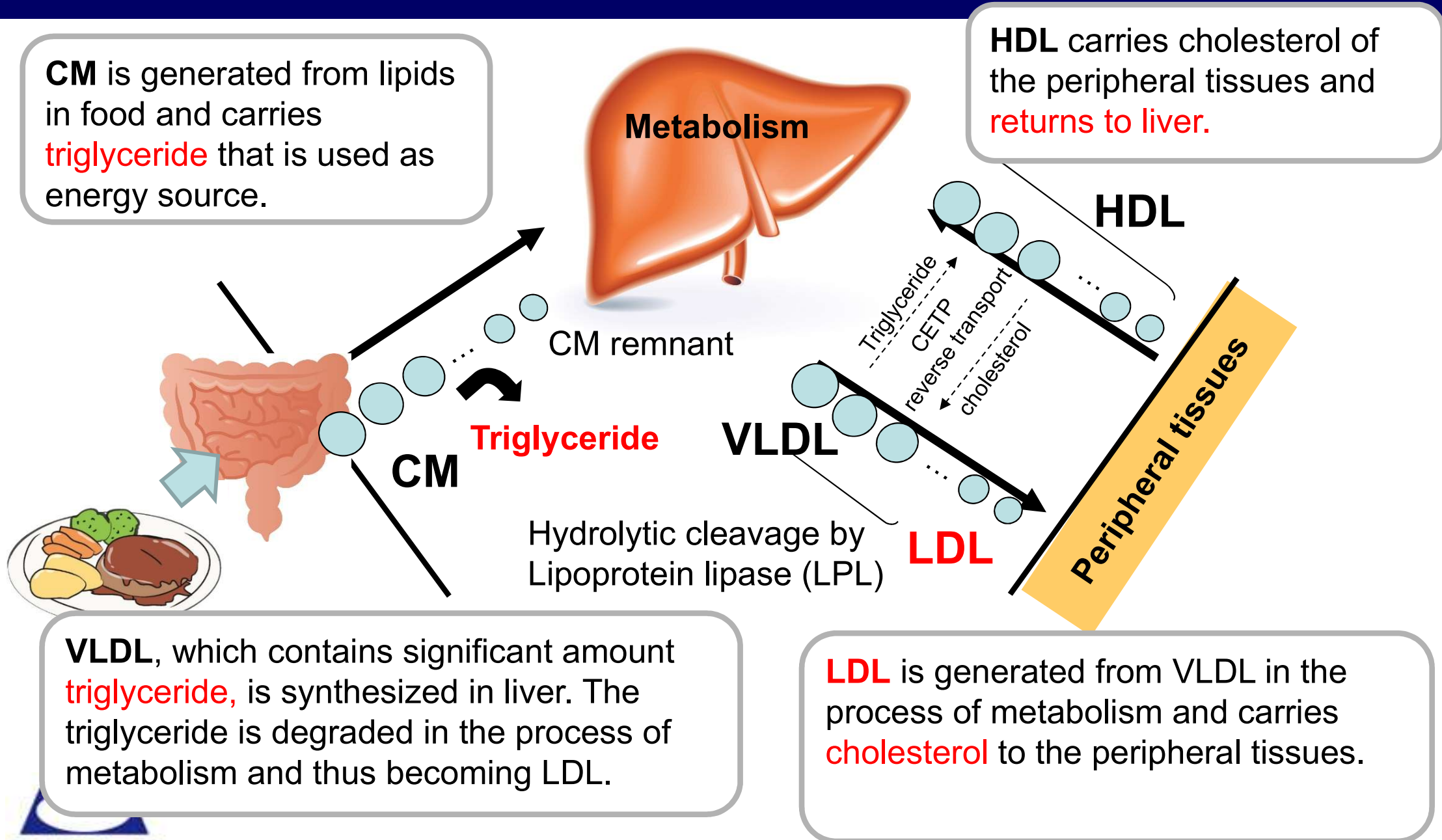
Thusly, **hydrophilic** carrier “Lipoprotein” is required to flow cholesterol and triglyceride in blood stream.

Classifications of Lipoprotein

4 Classification of Lipoprotein		CM	VLDL	LDL	HDL	
		Chylomicron	Very low density lipoprotein	Low density lipoprotein	HDL2	HDL3
Size (diameter, nm)		>70	30~70	15~30	~15	~7
Density		< 0.96	0.96 – 1.006	1.019 – 1.068	1.068 – 1.125	1.125 – 1.21
Lipid compositions (%)	TG	85	55	10	5	4
	CE	5	12	37	18	12
	FC	2	7	8	6	3
	PL	6	18	22	29	23
Proteins contained		2	9	23	42	58
Apolipoprotein compositions		Apo-I, ApoB-48, ApoC	ApoB-100, ApoC, ApoE	ApoB-100	Apo-I, Apo-II, ApoC, ApoE	Apo-I, Apo-II, ApoC

Lipoprotein Metabolism

CM is generated from lipids in food and carries **triglyceride** that is used as energy source.

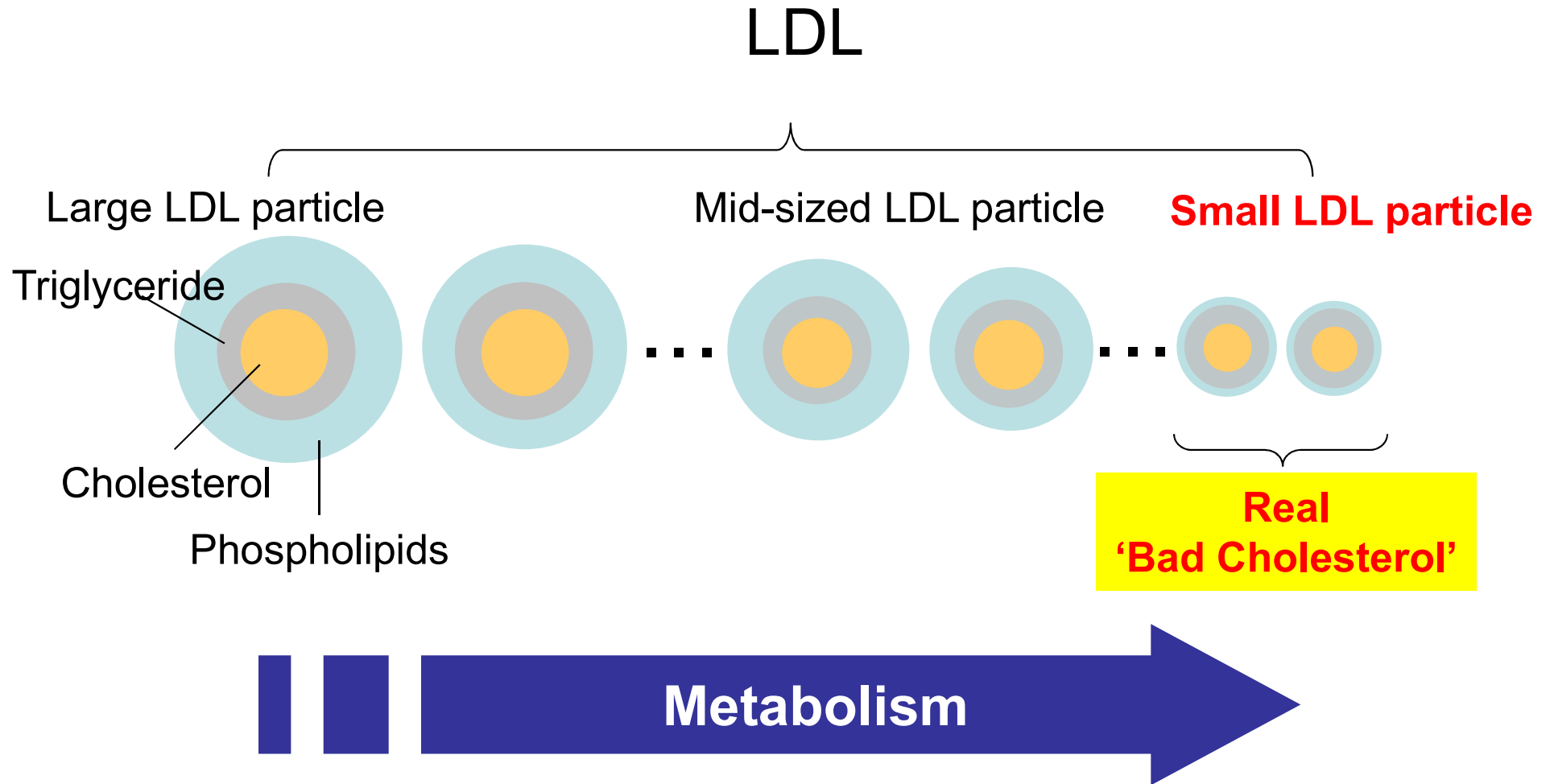


VLDL, which contains significant amount **triglyceride**, is synthesized in liver. The triglyceride is degraded in the process of metabolism and thus becoming **LDL**.

LDL is generated from **VLDL** in the process of metabolism and carries **cholesterol** to the peripheral tissues.

HDL carries cholesterol of the peripheral tissues and **returns to liver**.

What is the Most Risky Lipoprotein for Cardiovascular Event?



It is suggested that the smaller LDL is the most atherogenic and “Super bad.”

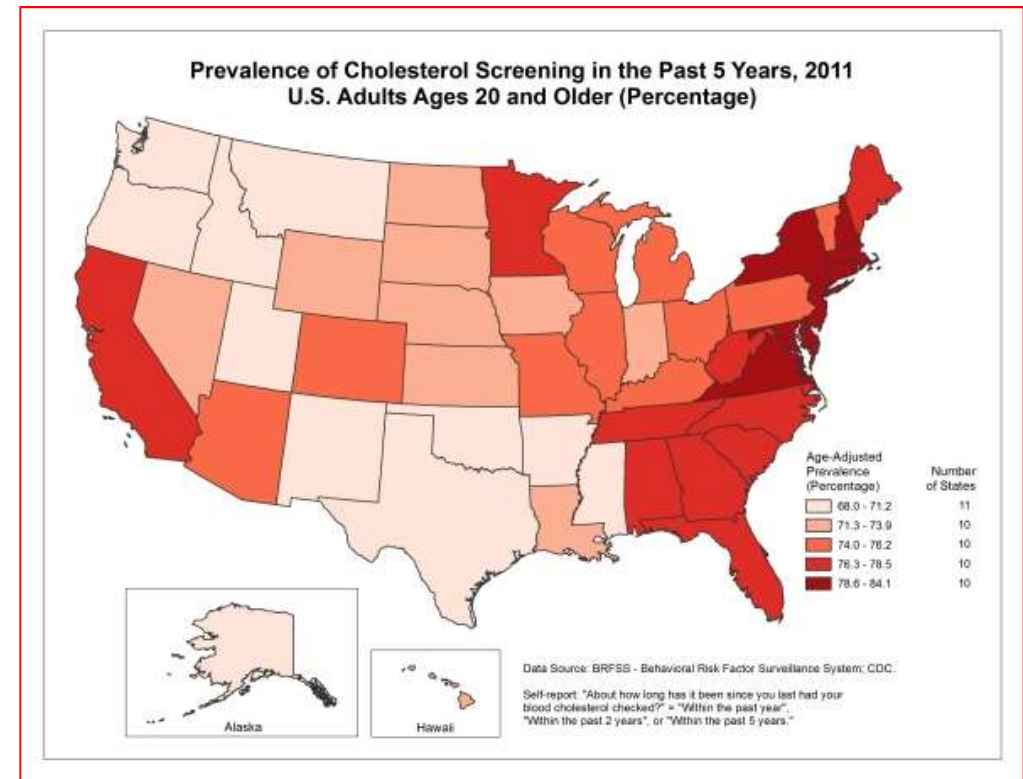
High LDL Population in EU and USA

Prevalence of high LDL in EU:
133 M people in 2014

Prevalence of high LDL in USA:
71 M people (33.5%) in 2011



Information Resource: EURATIV.com
<http://www.euractiv.com/section/health-consumers/infographic/infographic-europe-has-the-highest-prevalence-of-high-cholesterol-in-the-world/>



Information Resource: Centers for Diseases Control and Prevention DFD
http://www.cdc.gov/dhdsr/data_statistics/fact_sheets/fs_cholesterol.htm

99 M people in 2013



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