Separation of human plasma lipoproteins by density gradient centrifugation in iodixanol

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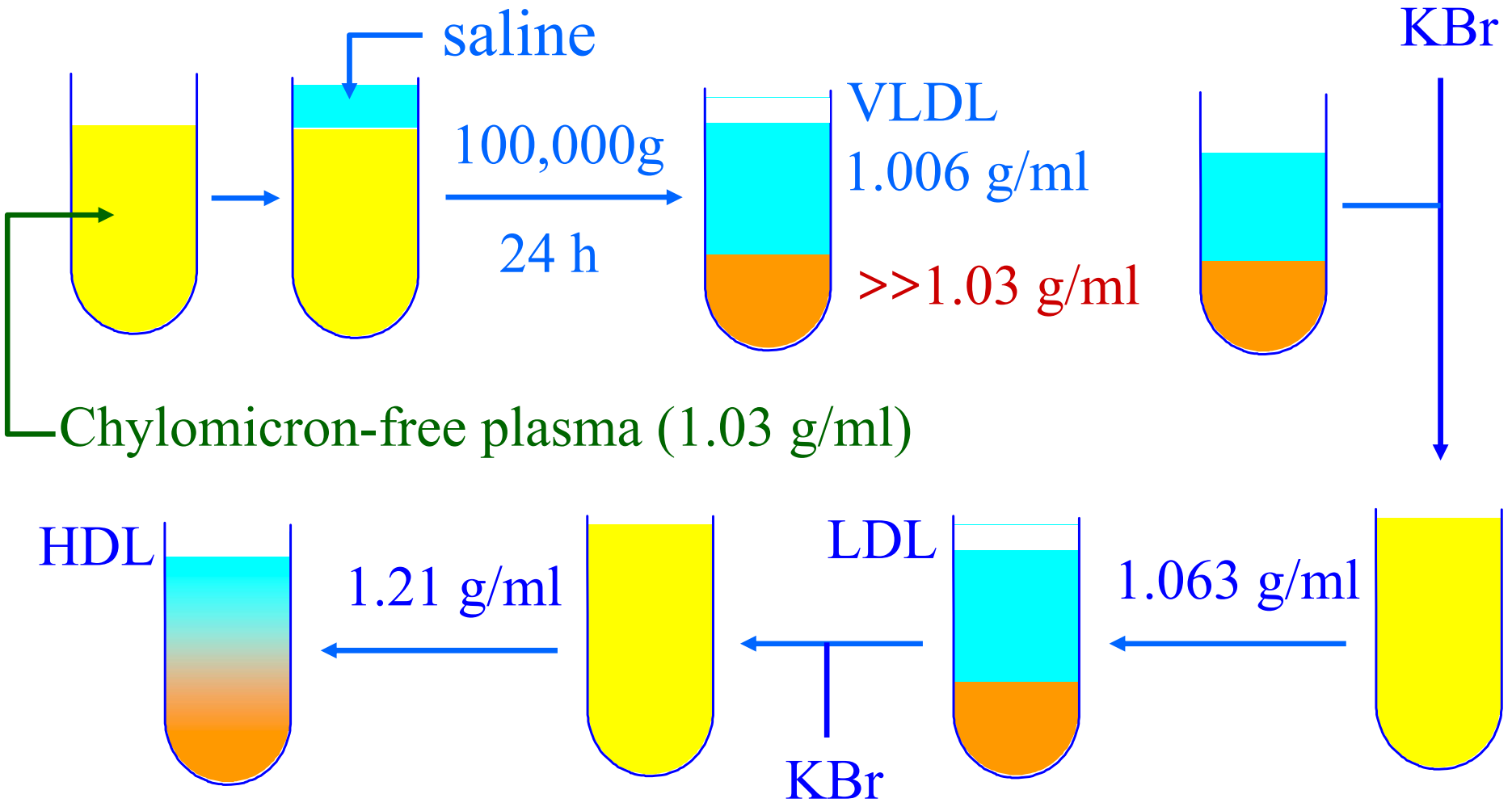
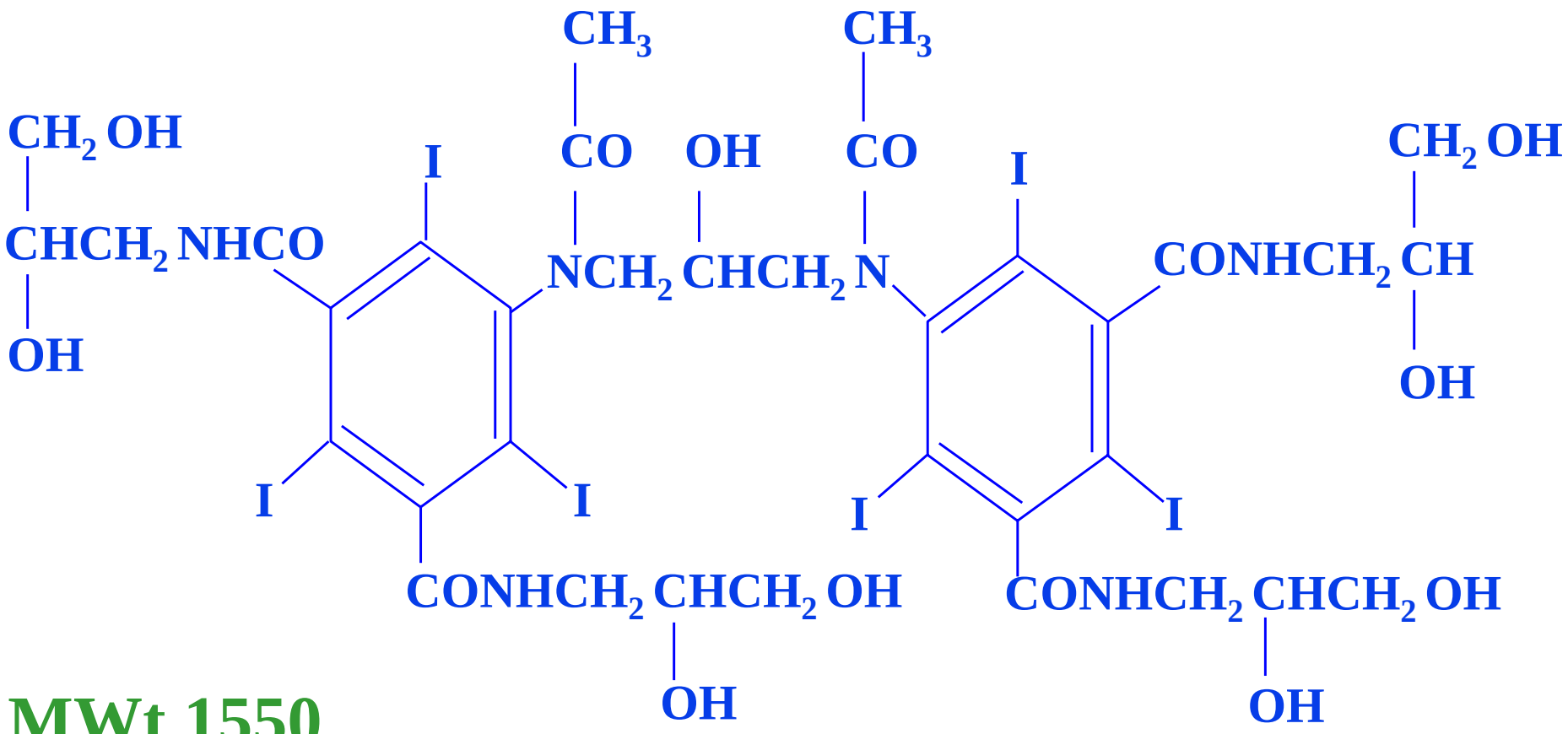
Composition and density of human plasma lipoproteins

Name	Density (g/ml)	Approx composition (% dry weight)			
		TAG.	Chol.	PL.	Prot.
Chylomicron	<0.95	80-95	2-5	3-6	1-2
VLDL	0.95-1.006	40-80	10-40	15-20	5-10
LDL	1.006-1.063	10	45	20	25
HDL	1.063-1.21	1-5	20	30	45-50

Fractionation of human plasma lipoproteins by sequential flotation



Iodixanol - a medium for self-generated density gradients



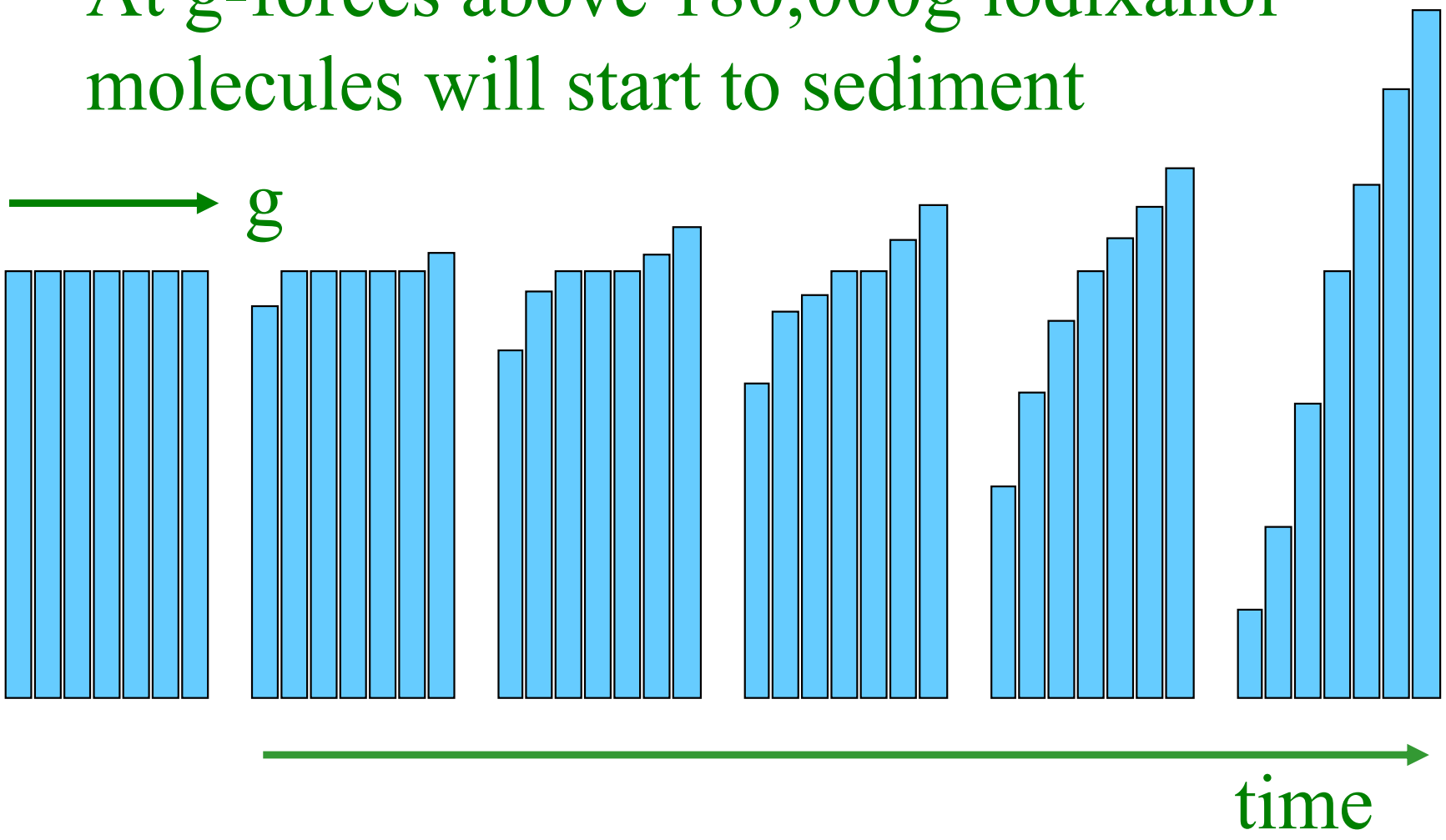
MWt 1550

Properties of iodixanol

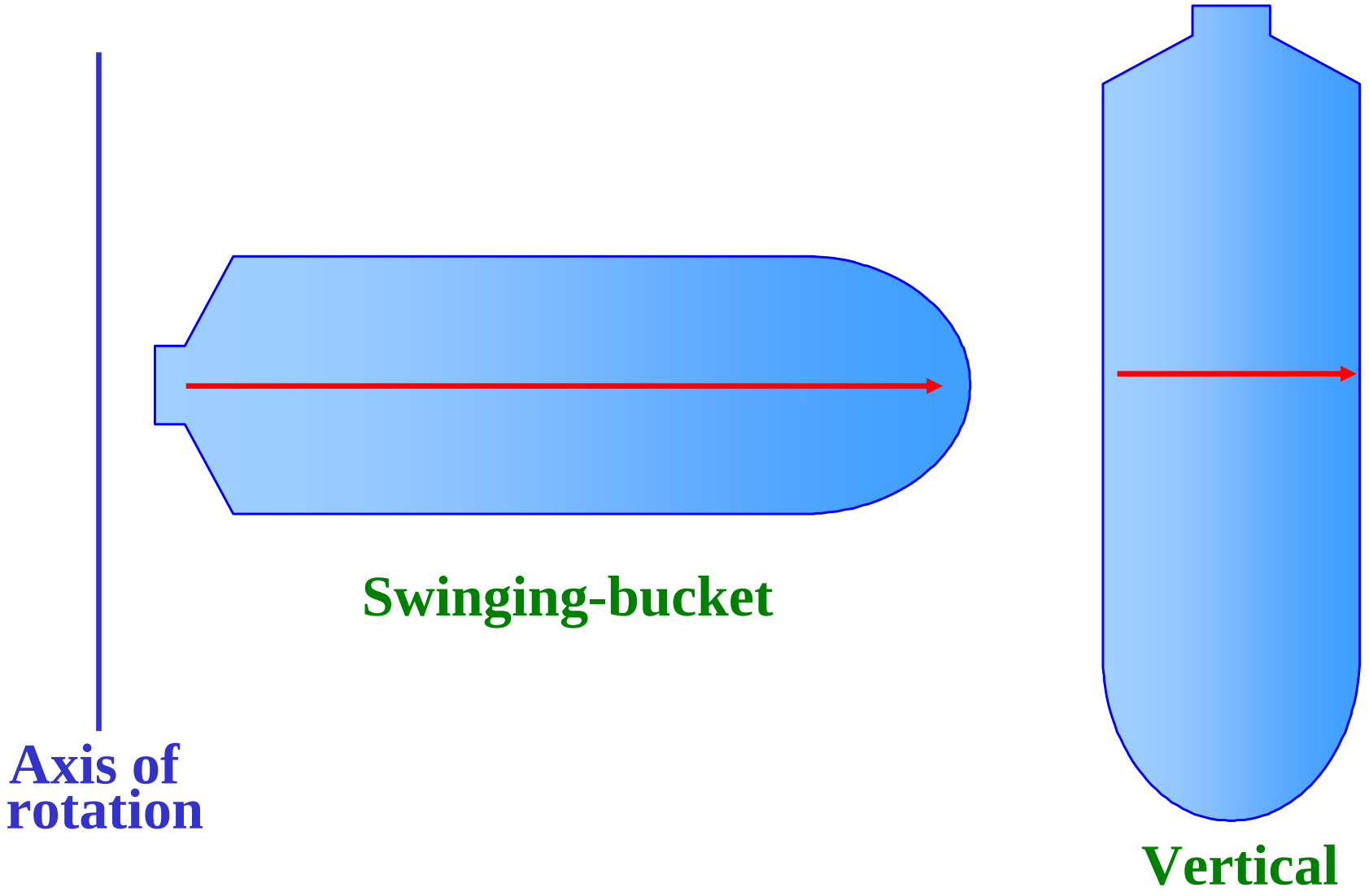
- Non-ionic
- Non-toxic
- Clinically tested as an X-ray imaging agent
- Commercially available as a 60% solution - OptiPrep™

Self-generated gradients

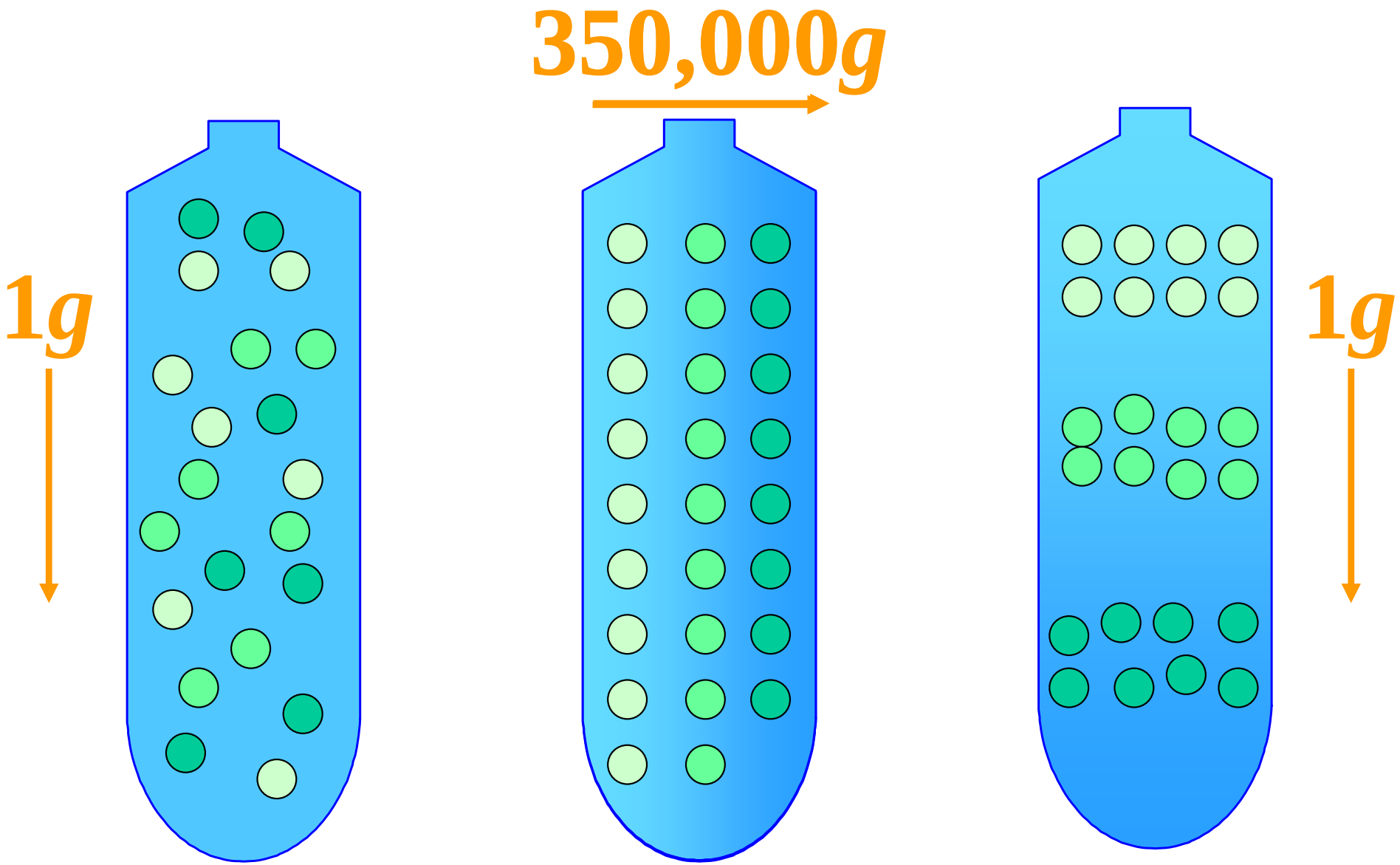
- At g-forces above 180,000g iodixanol molecules will start to sediment



Sedimentation path length of rotors



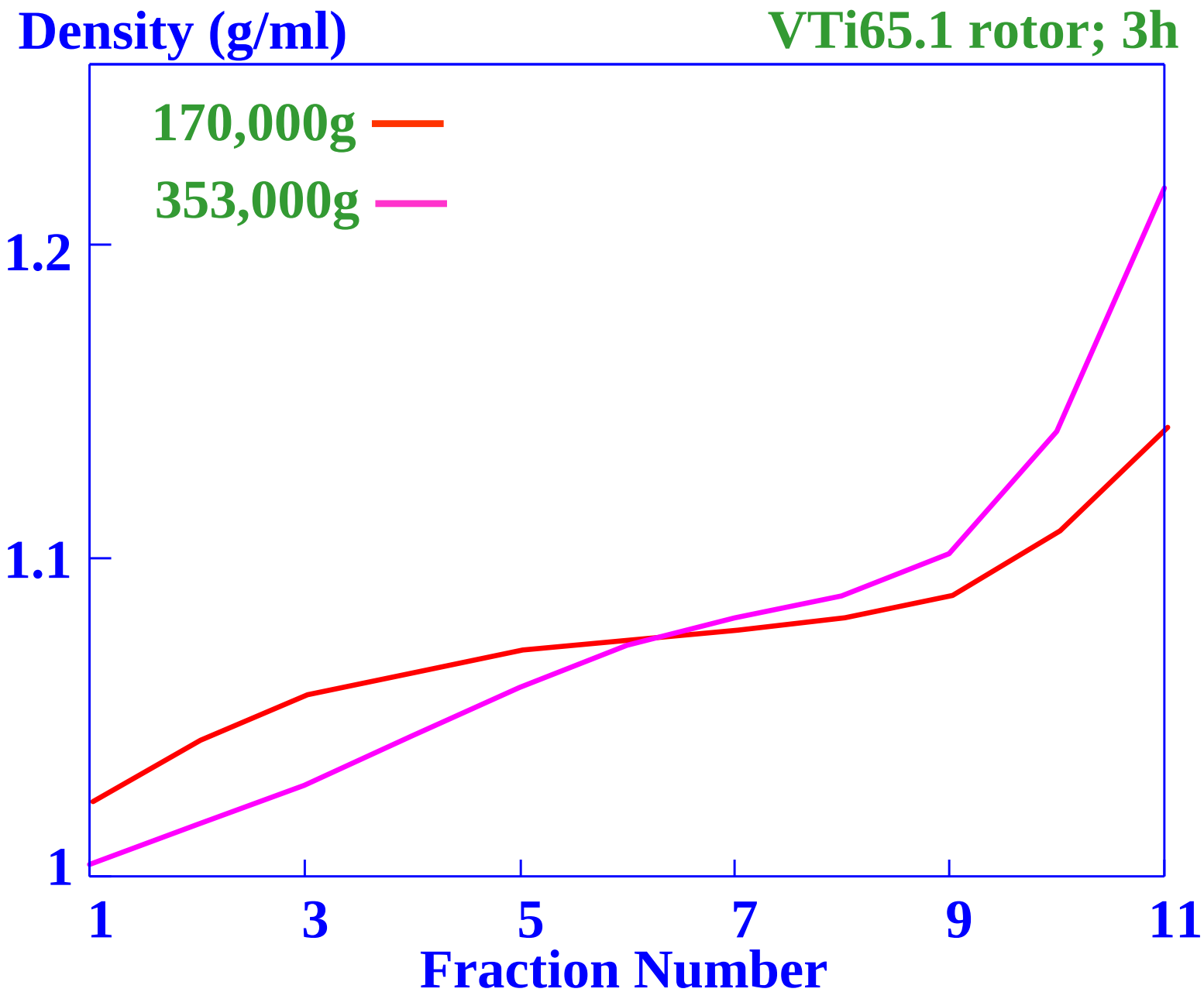
Self-generated gradient strategy



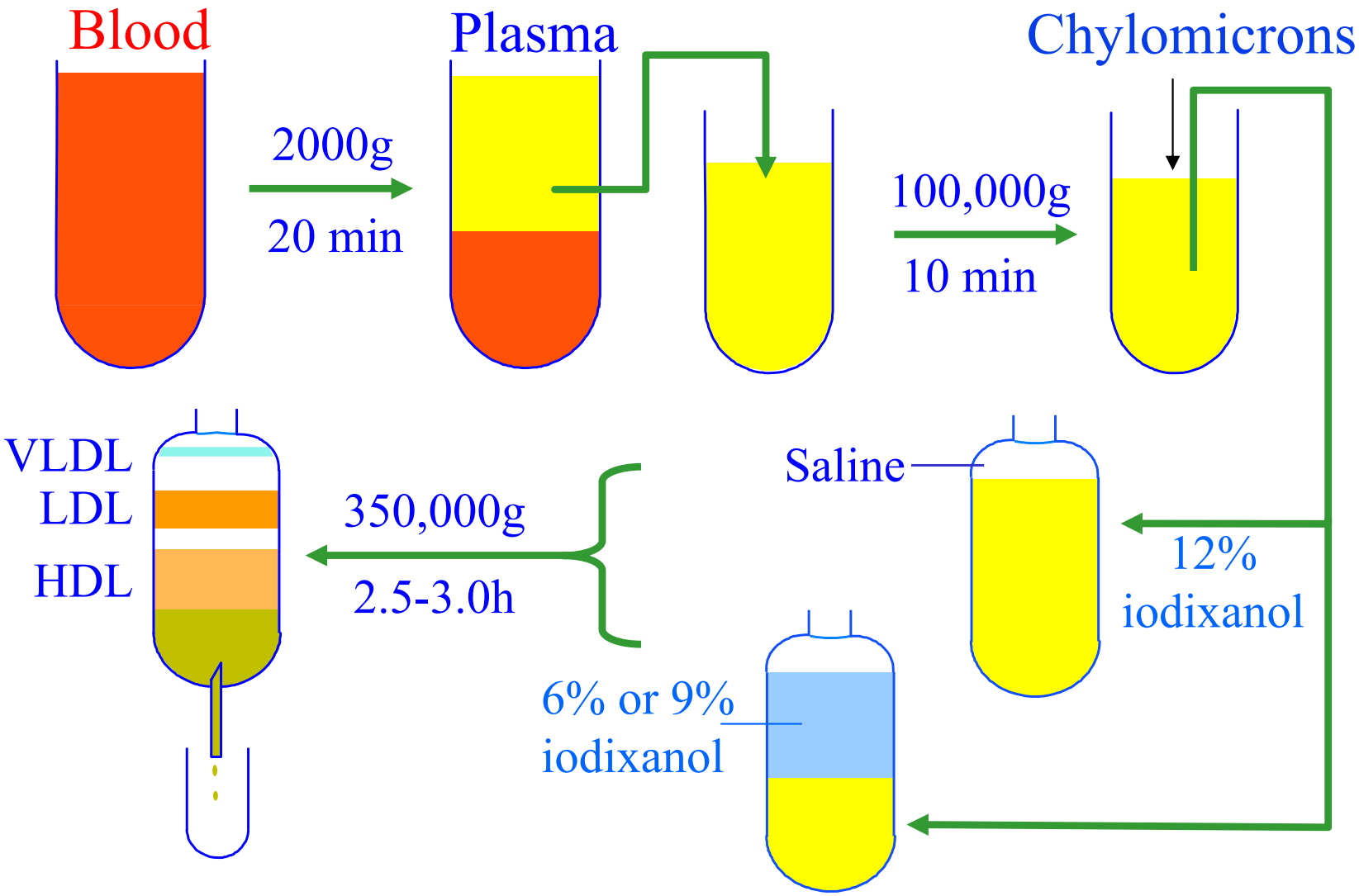
Common Beckman near-vertical and vertical rotors

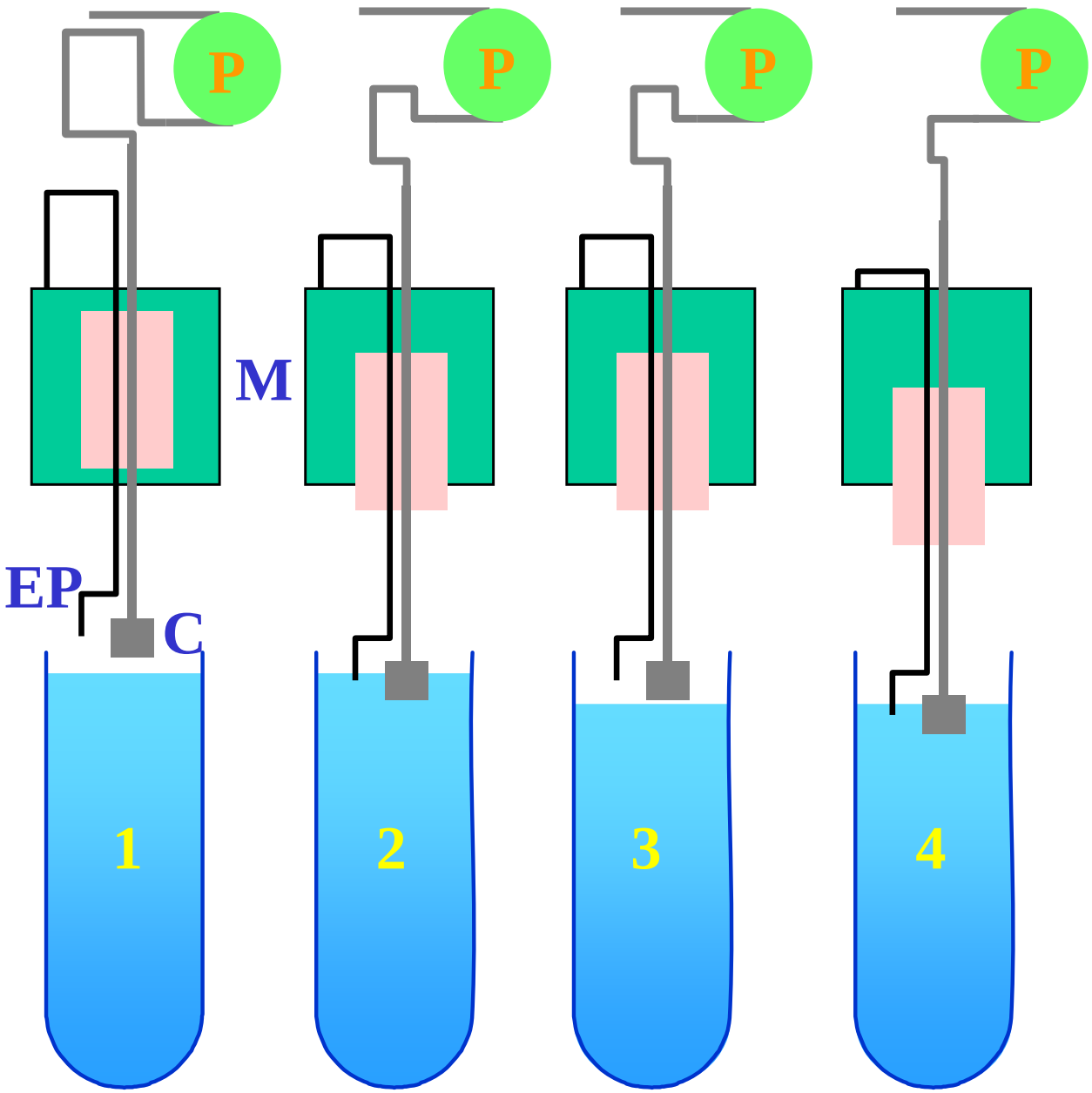
Rotor	Capacity	Maximum g
VTi90/NVT90	8 x 4.9ml	645,000
VTi65.2/NVT65.2	16 x 4.9 ml	416,000
VTi65.1/NVT65	8 x 11.2 ml	402,000
VTi50	8 x 36.2 ml	242,000
TLN100	8 x 3.3 ml	450,000
TLV100	8 x 2.0 ml	400,000

Start: 12% iodixanol (1.08 g/ml)



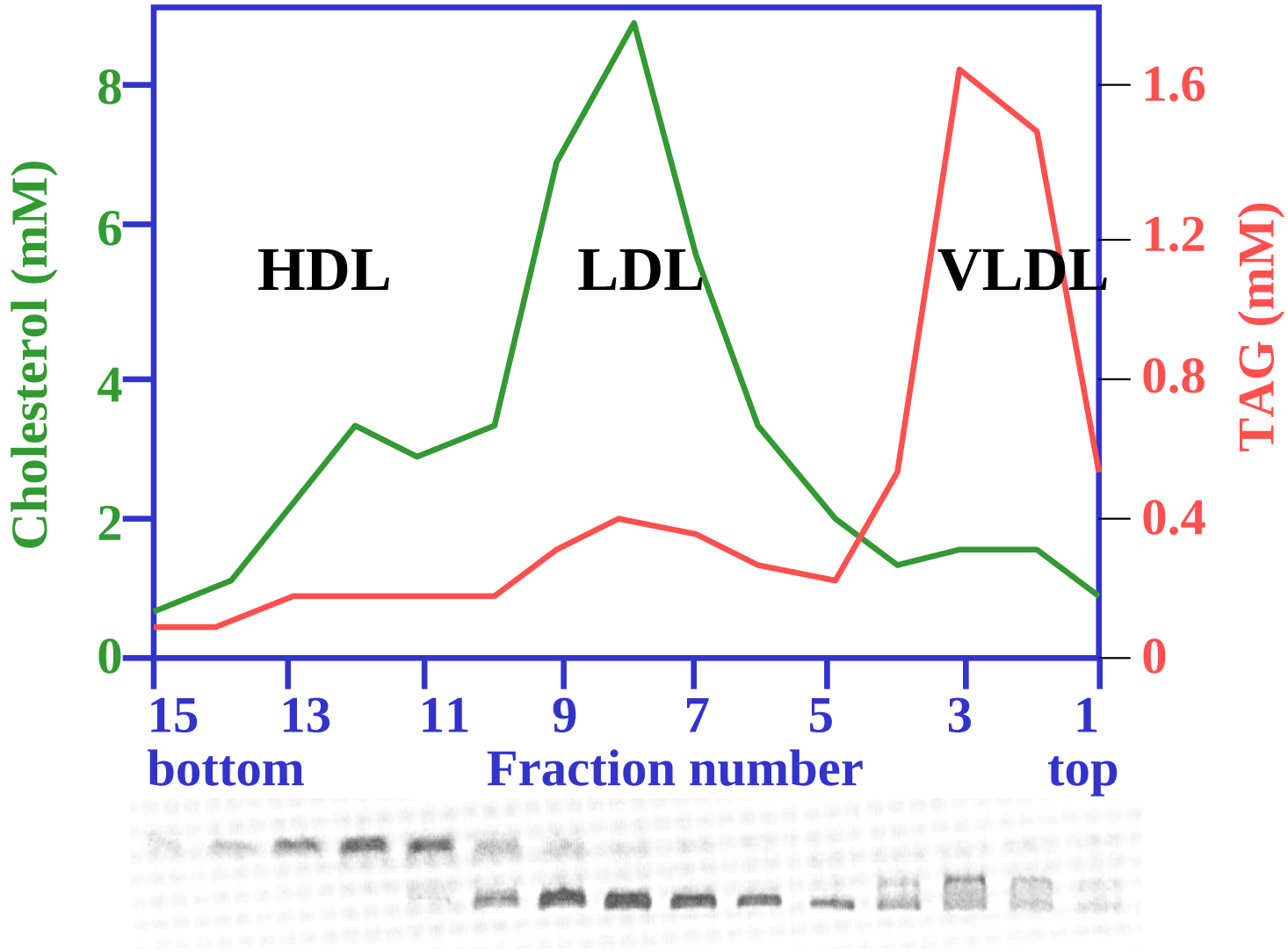
Lipoprotein fractionation strategy





Unloading density gradients with a Labconco Auto Densi Flow

Typical fractionation in 12% iodixanol in a TLN100 rotor



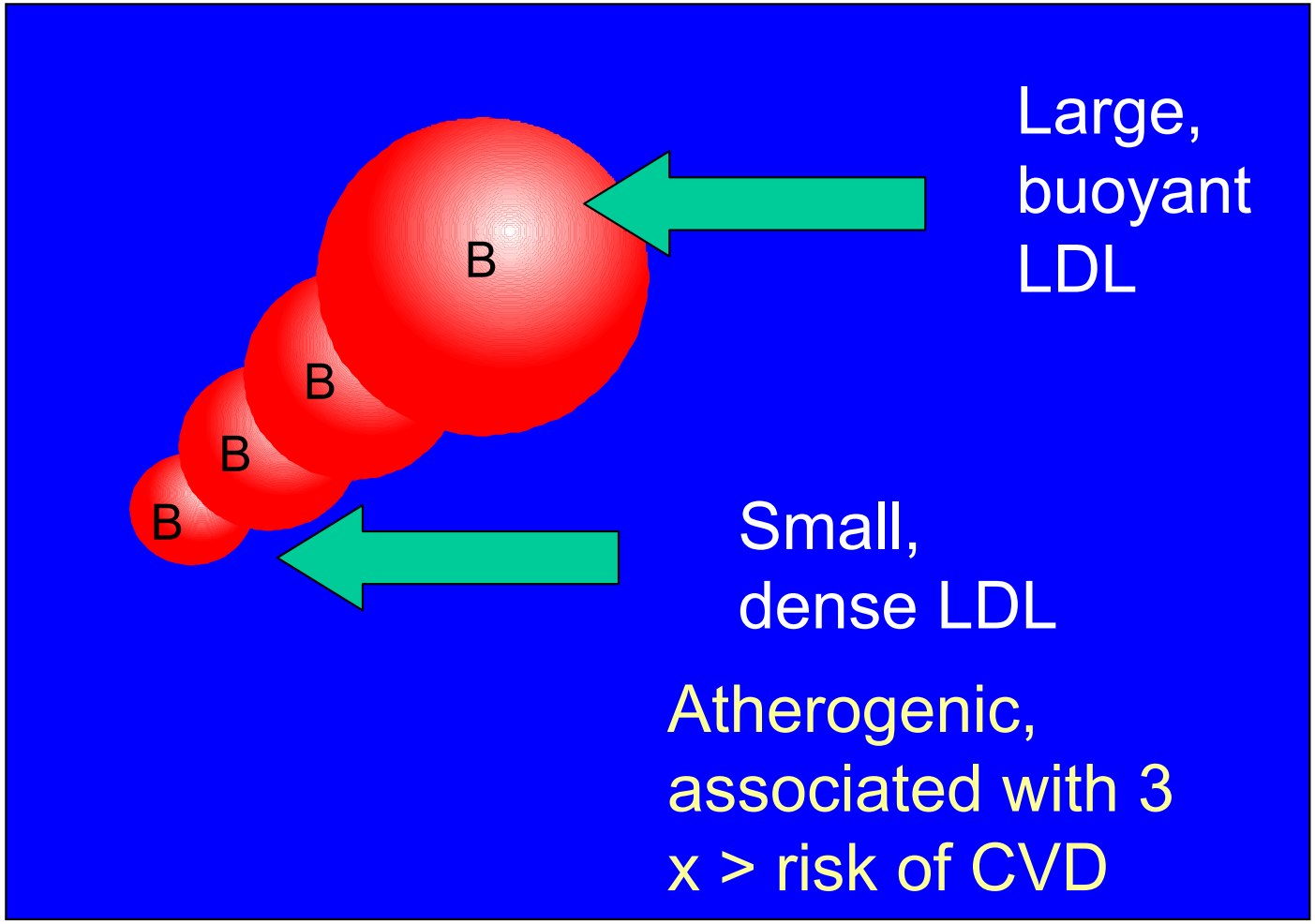
From: Higgins JA, Graham, JM & Davies I (2001) *In Atherosclerosis, Experimental Methods and Protocols* (ed Drew AF) Humana Press, Totowa, NJ pp 37-49.

Banding Densities in Iodixanol

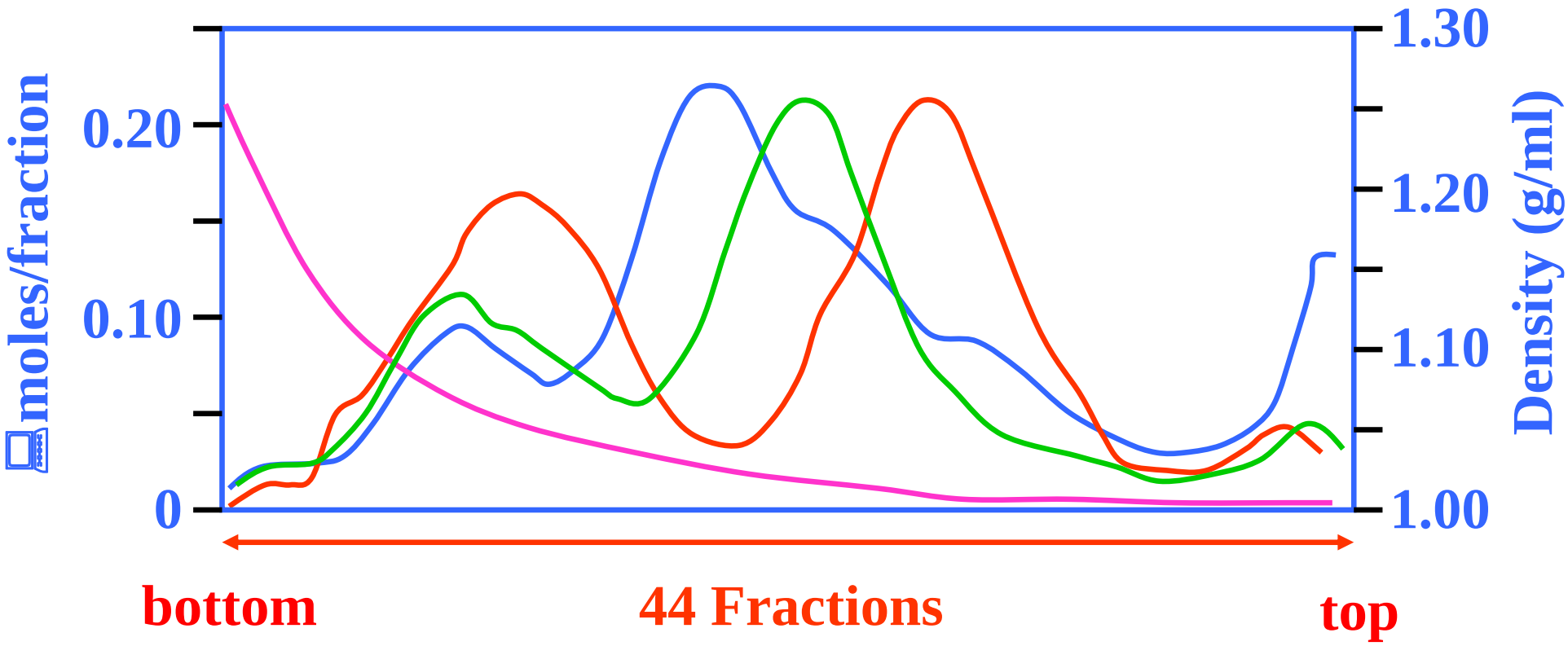
Lipoprotein	Density in KBr (g/ml)	Density in iodixanol (g/ml)
VLDL	<1.006	<1.006
LDL	1.019-1.063	1.01-1.03
HDL	1.063-1.210	1.03-1.14

Small dense, atherogenic LDL

LDLs consist of lipid and protein and range from a small dense, protein-rich particle to a more buoyant, lipid-rich one.

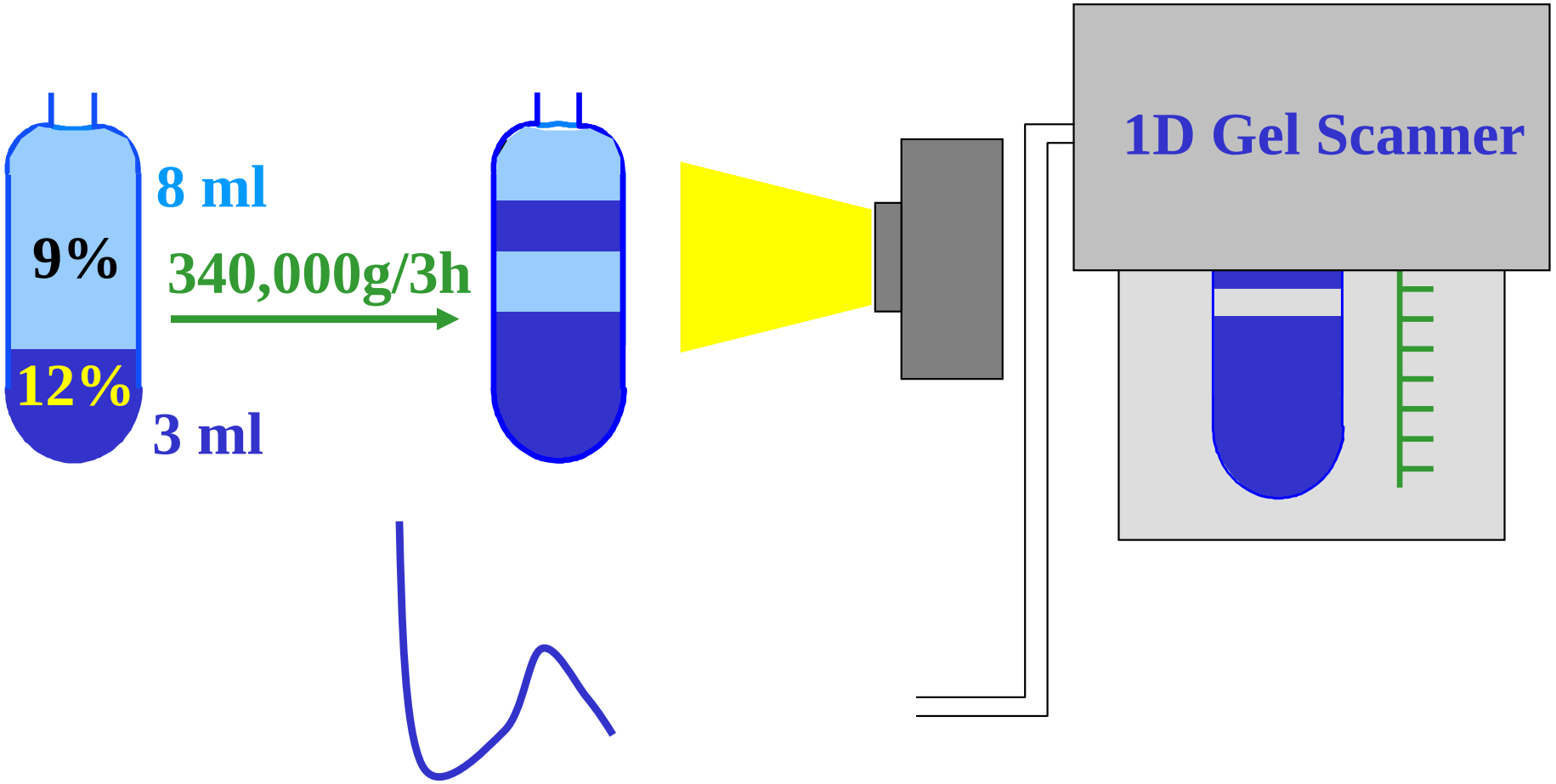


Cholesterol profiles of three subjects in 9/12% iodixanol self-generated gradients in TLN100 rotor



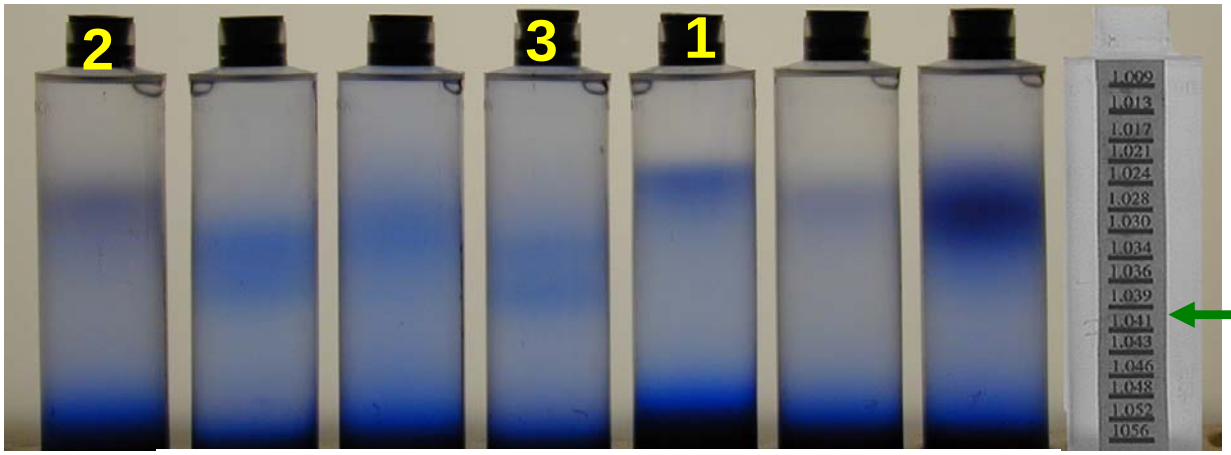
From Sawle, A, Higgins, MK, Olivant, MP,and Higgins, JA (2002)
J. Lipid Res. **43**, 335-343.

Fractionation and analysis of Coomassie blue stained lipoproteins in NVT65 rotor

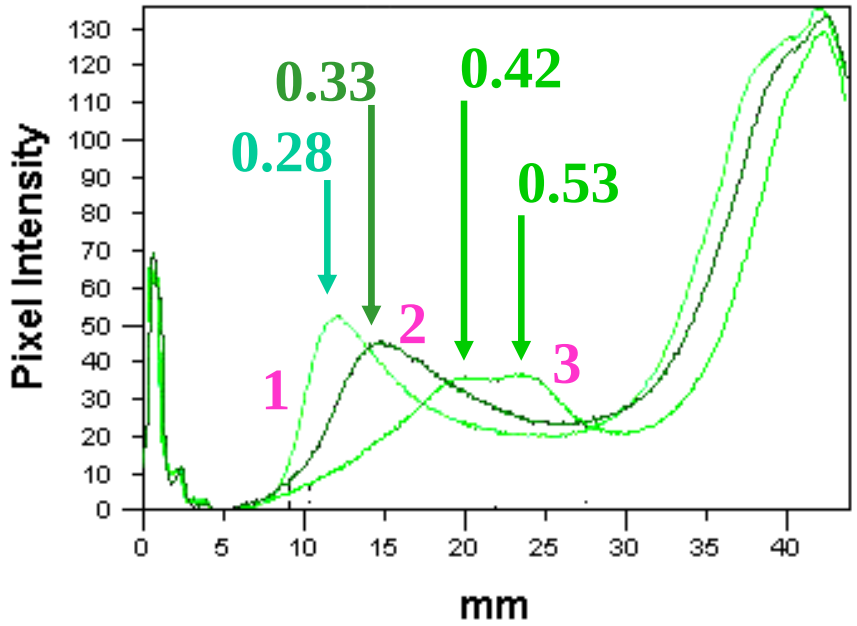


From Davies, IG and Griffin BA (2001) British Hyperlipidaemia Association Edinburgh, July 2001; *Atherosclerosis* (2001) **159**, 247-252.

Analysis of Coomassie-blue stained LDL bands

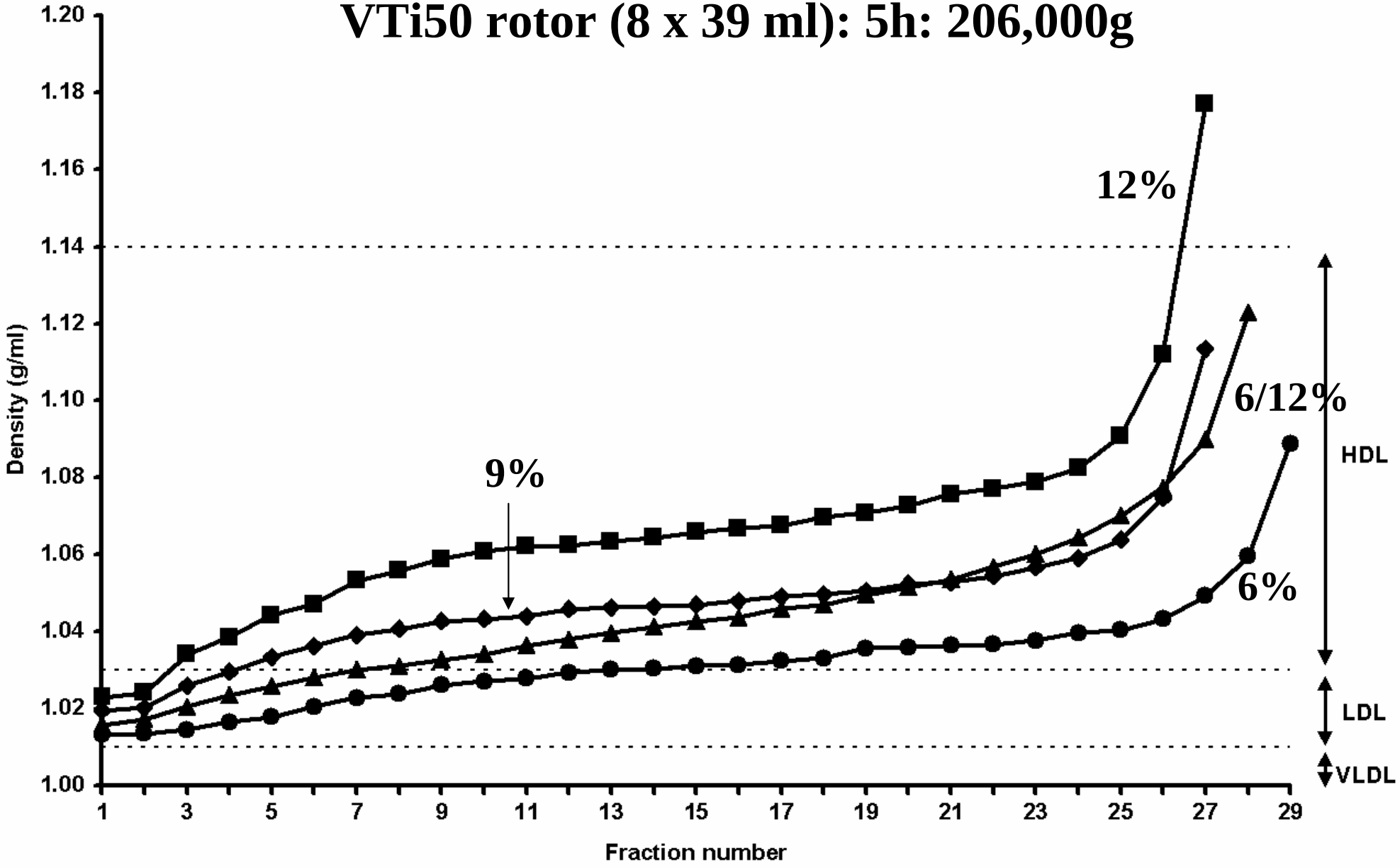


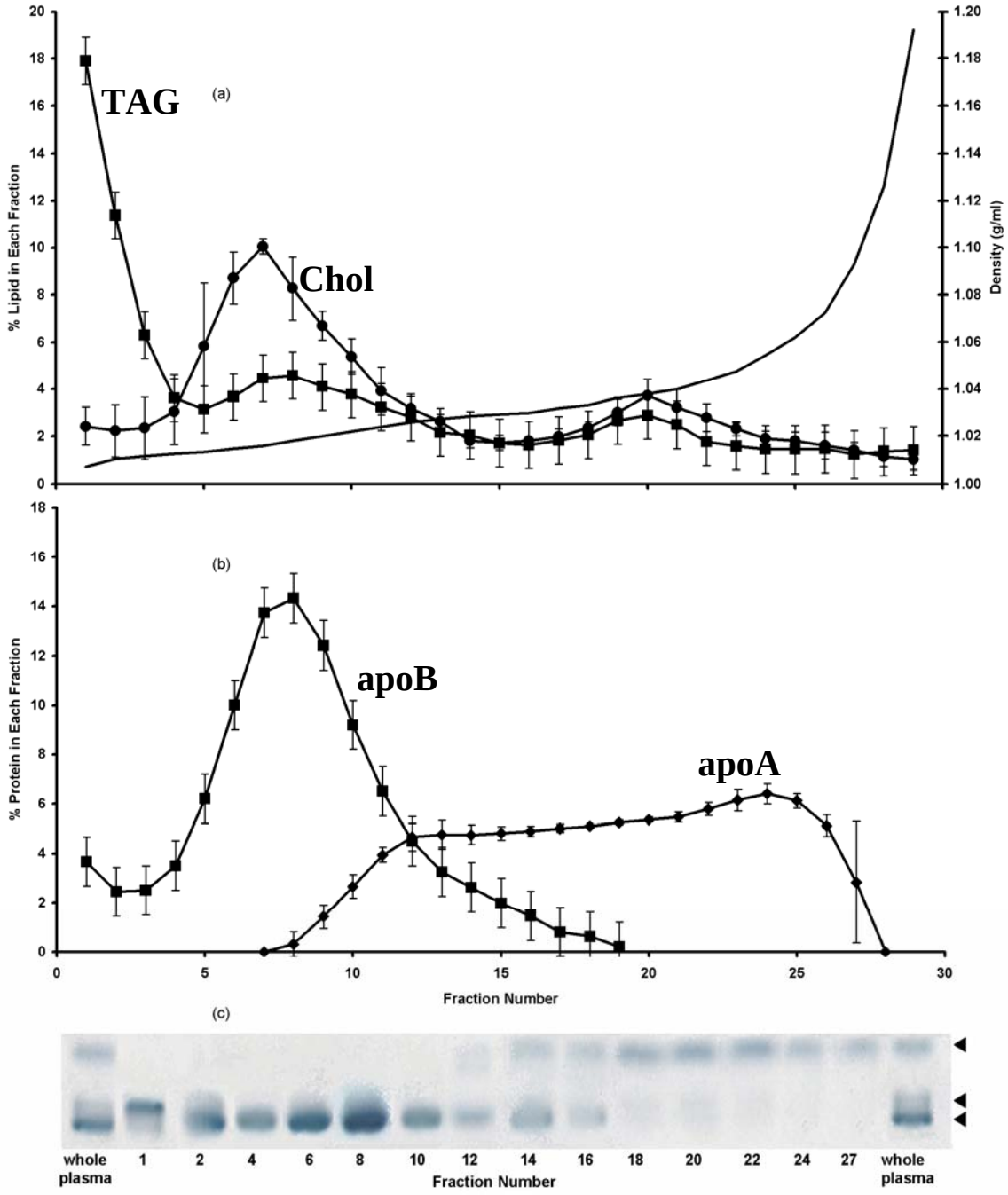
Density Reference scale



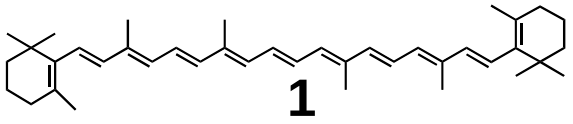
From Davies, IG and Griffin BA (2001) British Hyperlipidaemia Association Edinburgh, July 2001; *Atherosclerosis* (2001) **159**, 247-252.

Large Scale Preparation of lipoproteins



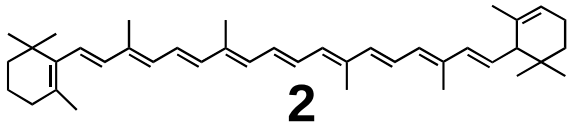


Separation of plasma lipoproteins in a VTi50 rotor



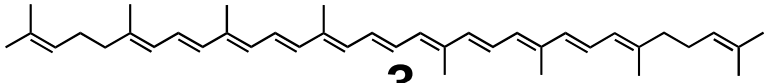
1

β-carotene



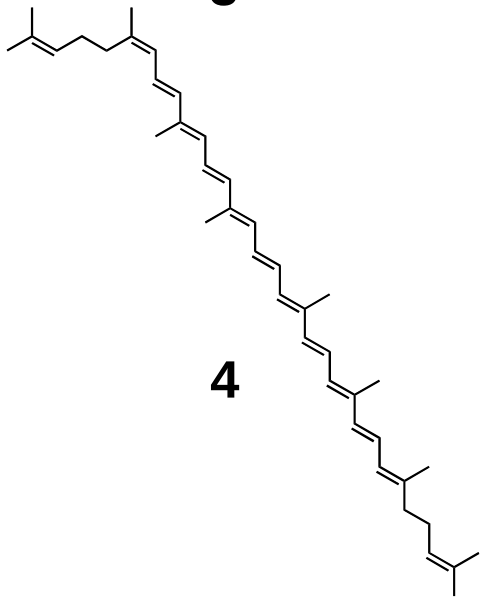
2

α-carotene



3

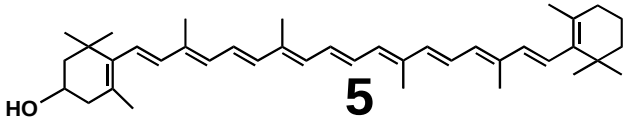
all-trans lycopene



4

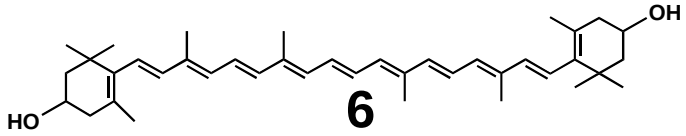
5-cis lycopene

Carotenes



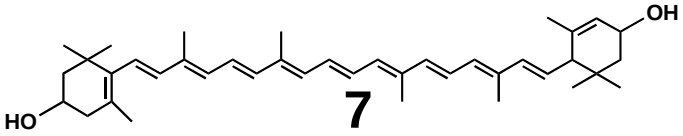
5

□-cryptoxanthin



6

zeaxanthin



7

lutein

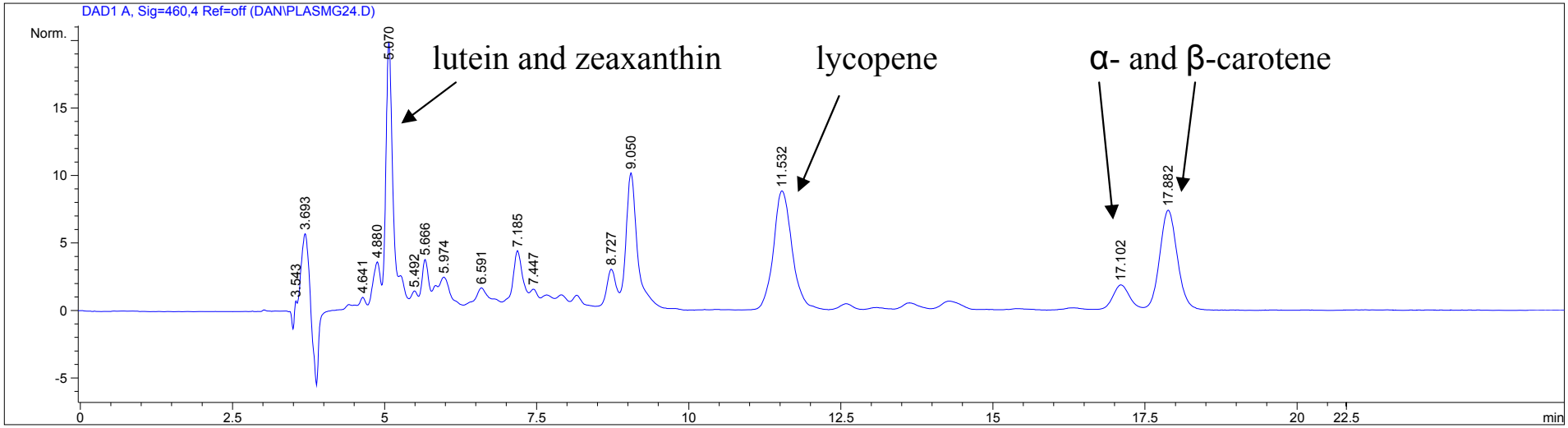
X Xanthophylls

Separation of plasma carotenoids on C18 column:

Source of extract : 1ml of plasma from female, aged 41, non-smoker

Extraction : 1ml ethanol, vortex, 1.5ml ethylt ether, vortex, 1.5ml hexane, vortex, take off top layer

Sample : dried under OFN at Rt and resuspended in 200µl THF/Methanol 10/90



System : Agilent 1100 Series LC Chemstation

Column : Phenomenex Gemini 5µm C18 110A 4.6 x 250mm

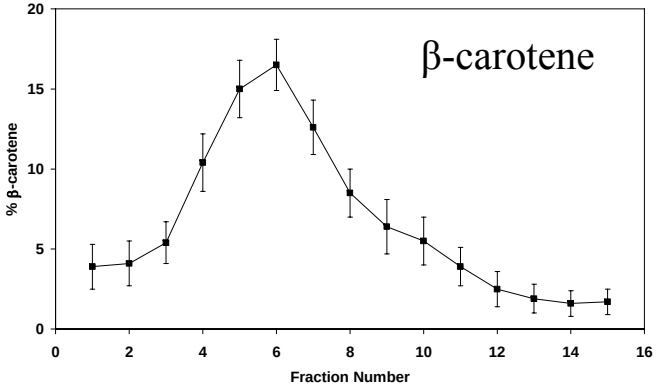
Mobile Phase : 66/22/10 Acetonitrile/THF/Methanol (with ammonium acetate)

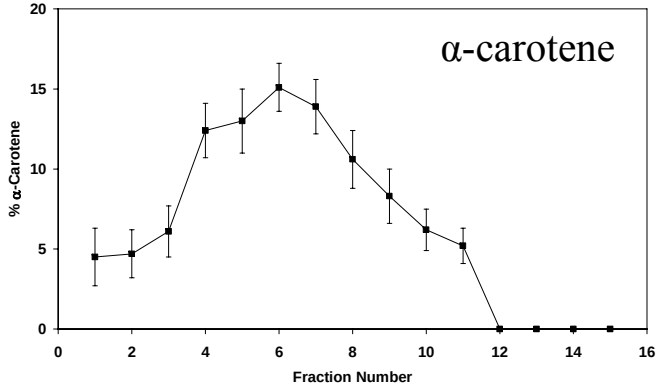
Temp : 22°C

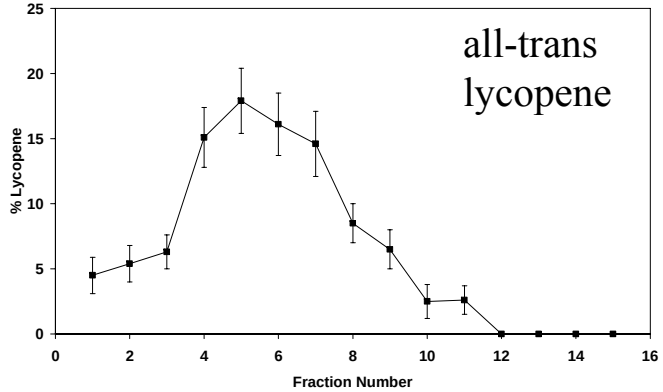
Flow Rate: 0.8 ml/min

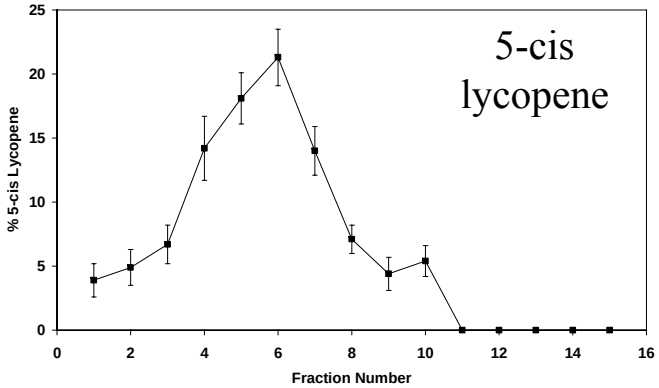
Injection Volume : 30µl

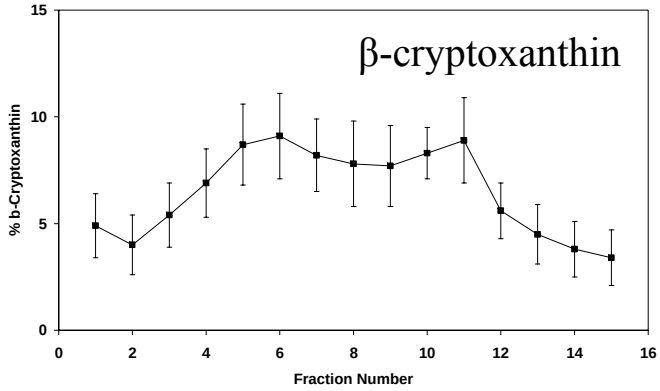
Carotenoid distribution in lipoprotein fractions

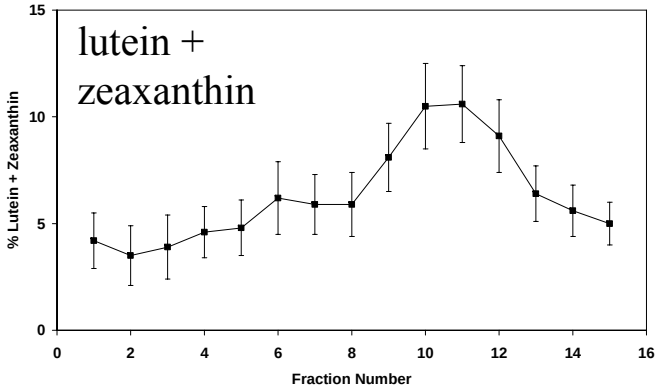




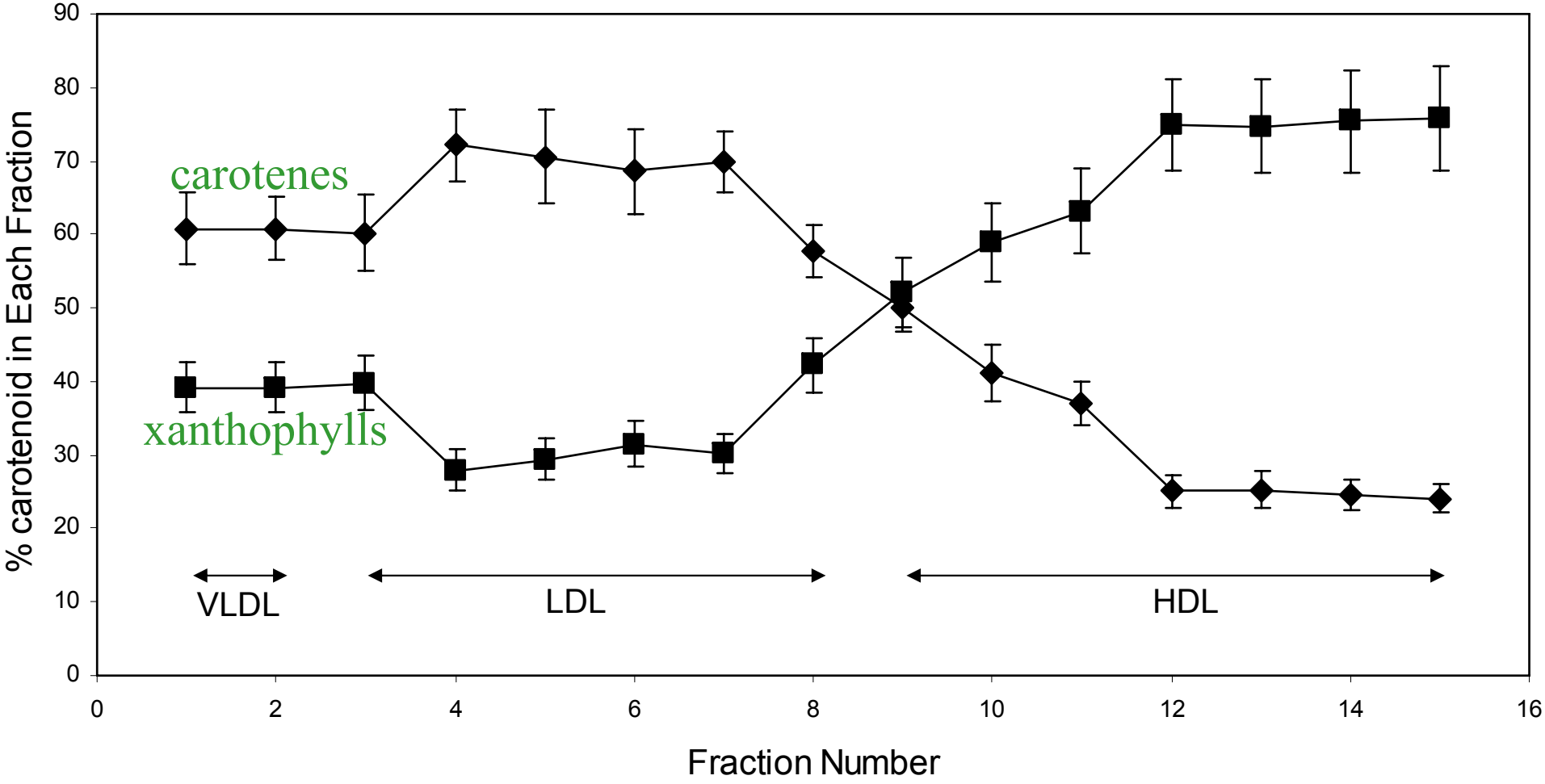




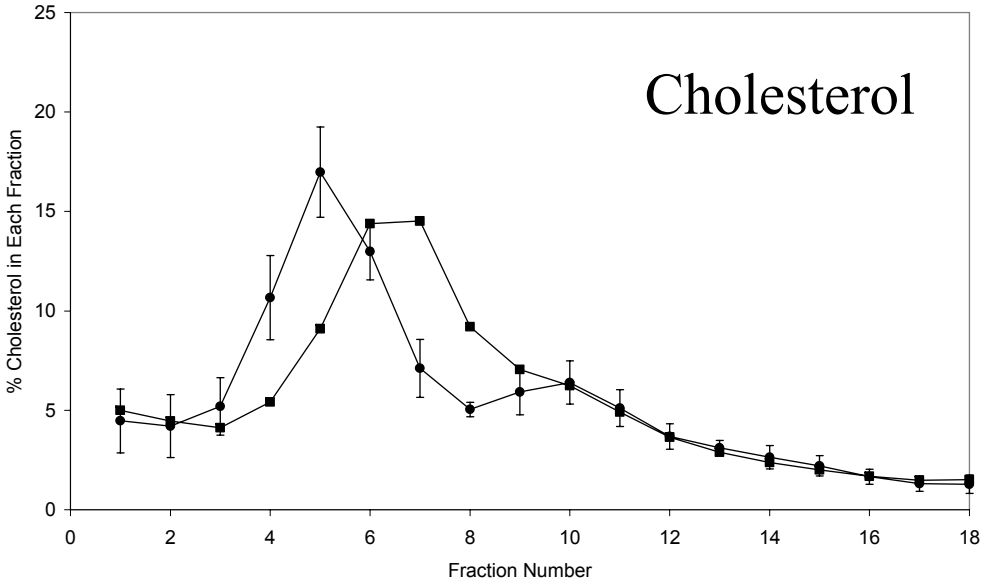


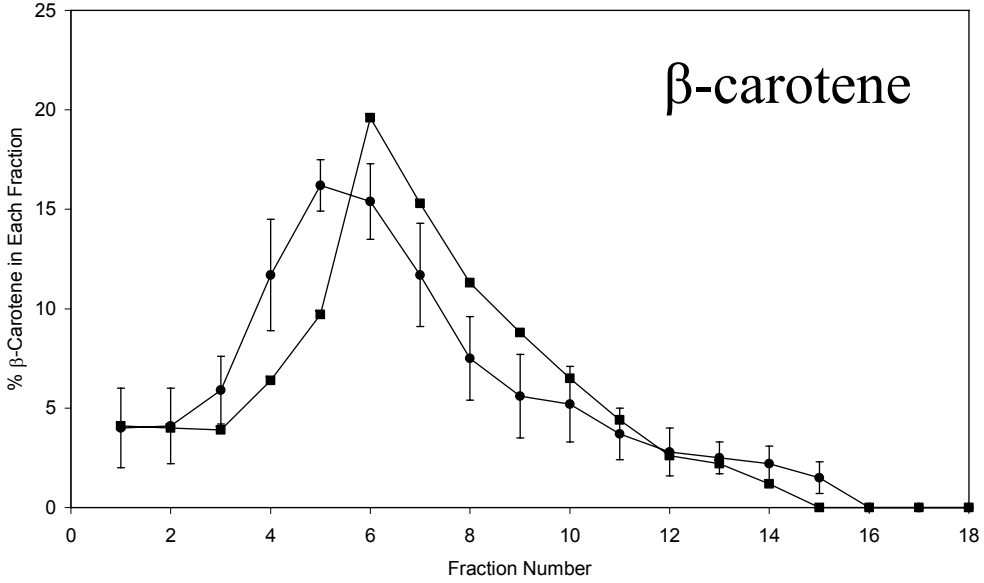


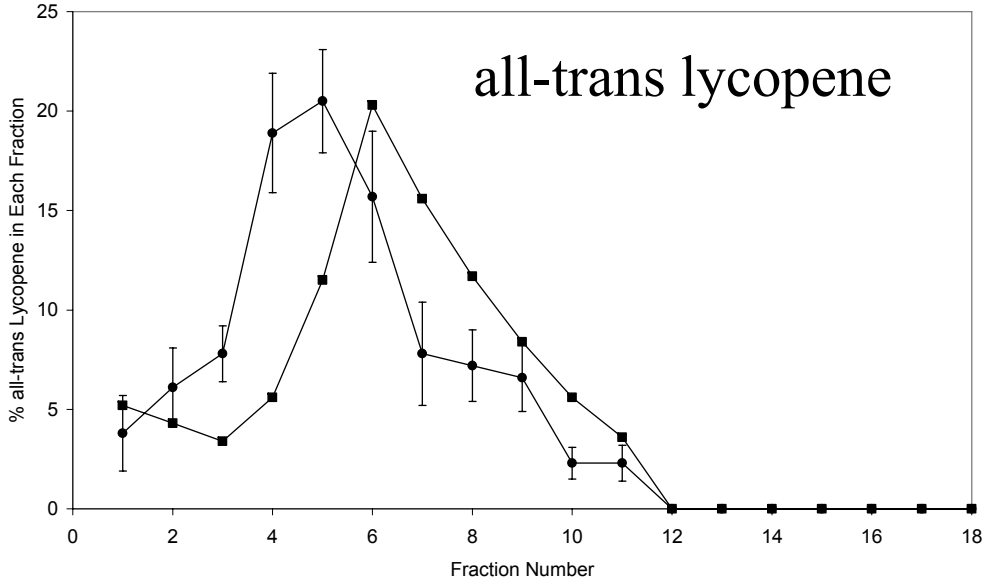
Carotenes are preferentially located in LDL and VLDL
Xanthophylls are preferentially located in HDL

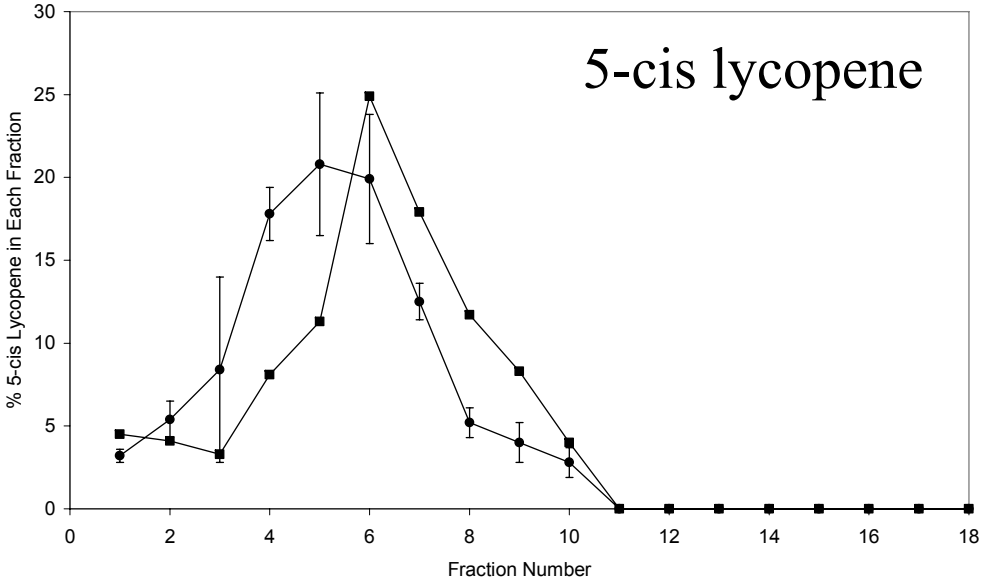


Small dense LDL particles are not depleted of carotenes









Advantages of iodixanol

- Lipoproteins close to their native state
- Self-generated gradients simple and reproducible
- Close to 100% recovery of lipids
- Identification and quantitation of the principal LDL subclasses and their components
- Potential for adaptation of techniques to HDL and VLDL subfractionation

With thanks to:

- Gordon Lowe
- Ian Davies
- John Graham and Terry Ford
- Andy Young and Danny Graham

(in no particular order)