OptiPrep™ Mini-Review MM01

Analysis of plasma lipoproteins – a bibliography

- This OptiPrep™ Mini-Review contains a brief summary of the methodology for the isolation and analysis of these particles (Section 1); the main aim of this Mini-Review however is to provide, in Section 2, a comprehensive list of references that report the use of OptiPrep™ for the fractionation of VLDL, LDL and HDL and also LDL and HDL sub-classes. The reference list (Section 1c) at the end of Section 1 applies only to this section.

1. Methodological review

1a. Separation and analysis of VLDL, LDL and HDL

Although ultracentrifugation is regarded as the "gold standard" method for the fractionation of plasma lipoproteins, the traditional method of sequential flotation by incrementally increasing the density of the plasma with KBr to provide sequentially VLDL, LDL and HDL is technically simple but excessively tedious (requiring approx. 3 days). The alternative approach involves the use of either discontinuous or continuous KBr/NaCl gradients; these gradients are technically very difficult to produce and handle. Detailed descriptions of the methodologies can be found in references 1-5. Moreover use of some add-on analytical techniques often necessitates removal of the salt by dialysis, adding up to 12 h to the procedure. Moreover, the use of high salt concentrations may cause the loss of certain surface apoproteins from lipoproteins.

The introduction of self-generated gradients of iodixanol in 1996 [6,7] solved many of the problems associated with earlier technology. A simple one step centrifugation for 3 h that resolves VLDL, LDL and HDL, avoiding the use of technically-difficult salt gradients is summarized in Figure 1. Chylomicron-free plasma is simply mixed with OptiPrep™, transferred to tube for a near-vertical rotor; overlayered with a little saline to fill the tube and centrifuged for 3 h. During the centrifugation the shallow resolving gradient is formed and the lipoproteins move to their banding density. The gradient may be collected as shown in Figure 1 by tube puncture or by aspiration from the meniscus.

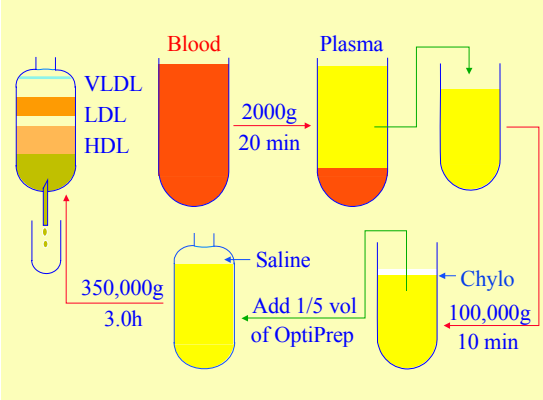

Figure 1: Fractionation of plasma lipoproteins in a self-generated iodixanol gradient from chylomicron-free plasma

1b. Separation and analysis of LDL subclasses

By a small modulation in the starting format, the gradient may be adapted to the fractionation of LDL subclasses; the plasma is adjusted to 12% (w/v) iodixanol (as recommended in refs 6 and 7) but it is overlayered by a solution of 9% (w/v) iodixanol. In a small volume near-vertical rotor such as the Beckman TLN100 the volumes of plasma/12% iodixanol and 9% iodixanol are equal [8]. In the larger volume NVT65 the volumes of 3 ml and 8 ml respectively allow a better resolution of the denser LDL from the HDL [9] and the low density region of the gradient is more shallow , allowing a greater linear separation of the LDL subclasses. In Figure 2 the (3ml + 8 ml) configuration is shown in conjunction with the use of a Coomassie blue stained plasma sample; this allows a density profile of the LDL band to be produced by scanning a digital photograph of the tube assessed without unloading the gradient or assaying the fractions for cholesterol and/or triacylglycerol.

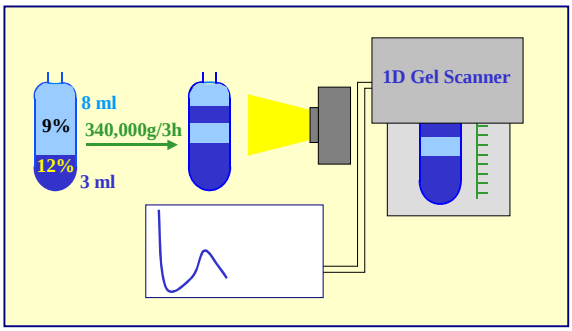

Figure 2: Fractionation and analysis of Coomassie-blue stained plasma lipoproteins in a self-generated iodixanol gradient

- This strategy has been adapted to the analysis of HDL subclasses in a Sudan black stained plasma [10]
- Detailed protocols for the purification and analysis of the VLDL, LDL and HDL and for the analysis of LDL subclasses (Application Sheets M07 and M08 respectively) may be accessed from the Axis-Shield OptiPrep™ Applications CD or from the following website: www.axis-shield-density-gradient-media.com, click on "Methodology" then "Macromolecules" to open up the "Macromolecules and Macromolecular Complex Index". Other OptiPrep™ Application Sheets on the preparation of self-generated gradients and harvesting of gradients may also be accessed from the top of the Index.

1c. References

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2. Bibliography

Section 2a lists those references reporting studies on plasma lipoproteins and is subdivided alphabetically into animal species (e.g. **2a-1 Black bear**). The vast majority of papers are concerned with human plasma lipoproteins (**2a-5**); this section is subdivided into those concerned with the major lipoprotein classes (**2a-5-1**), HDL subclasses (**2a-5-2**) and LDL subclasses (**2a-5-3**). Within Sections **2a-5-1** and **2a-5-2**, references are further divided according to research topic, listed alphabetically. A reference may appear in two or more of these research topic subsections. **Section 2b** lists references reporting the analysis of lipoproteins from sources other than plasma. Within all sections references are listed alphabetically according to **first author** and chronologically for multiple papers from the same first author. Key words in reference titles are highlighted in red.

2a Plasma lipoproteins

2a-1 Black bear

Frank, N., Elliott, S.B., Allin, S.B. and Ramsay, E.C. (2006) *Blood lipid concentrations and lipoprotein patterns in captive and wild American black bears (Ursus americanus)* Am. J. Vet. Res., **67**, 335-341

2a-2 Bovine

Gardner, R.S., Ogden, N.H., Cripps, P.J. and Billington, D. (2003) *Separation of bovine plasma lipoproteins by a rapid ultracentrifugation method* J. Comp. Path., **128**, 15-23

2a-3 Fish

Aas, G.H., Bjerkeng, B., Storebakken, T. and Ruyter, B. (1999) *Blood appearance, metabolic transformation and plasma transport proteins of ^{14}C-astaxanthin in Atlantic salmon (Salmo salar L.)* Fish Physiol. Biochem., **21**, 325-334

Aursnes, I.A.S., Gjoen,T. and Rishovd, A-L. (2009) *Effect of hyperthermia on plasma lipids and gene expression in Atlantic Cod (Gadus Morhua I.)* Toxicol. Lett., **189S**, S192
Magnoni, L. and Weber, J-M. (2007) *Endurance swimming activates trout lipoprotein lipase: plasma lipids as a fuel for muscle* J. Exp. Biol., **210**, 4016-4023
Prindiville, J.S., Mennigen, J.A., Zamora, J.M., Moon, T.W. and Weber, J-M. (2011) *The fibrate drug gemfibrozil disrupts lipoprotein metabolism in rainbow trout* Toxicol. Appl. Pharmacol., **251**, 201–208

2a-4 Hamster

Bennett, A.J., Kendrick, J.S., Anderton, K.L., Higgins, J.A. and White, D.A. (1997) *Effect of dietary fish oil or sunflower oil on plasma lipoproteins and hepatic gene expression in the hamster* Atherosclerosis, **130 (Suppl. 1),** S24

2a-5 Human

2a-5-1 HDL/LDL/VLDL

Acrolein effects

Conklin, D.J., Barski, O.A., Lesgards, J-F., Juvan, P., Rezen, T., Rozman, D., Prough, R.A., Vladykovskaya, E., Liu, S-Q., Srivastava, S. and Bhatnagar, A. (2010) *Acrolein consumption induces systemic dyslipidemia and lipoprotein modification* Toxicol. Appl. Pharmacol., **243**, 1–12

Apolipoprotein B100

Mason, R.P., Sherratt, S.C.R. and Jacob, R.F. (2016) *Eicosapentaenoic acid inhibits oxidation of apoB-containing lipoprotein particles of different size in vitro when administered alone or in combination with atorvastatin active metabolite compared with other triglyceride-lowering agents* J. Cardiovasc. Pharmacol., **68**, 33–40
Rabbani, N., Chittari, M.V., Bodmer, C.W., Zehnder, D., Ceriello, A. and Thornalley, P.J. (2010) *Increased glycation and oxidative damage to apolipoprotein B100 of LDL cholesterol in patients with type 2 diabetes and effect of metformin* Diabetes, **59**, 1038–1045

Astaxanthin

Coral-Hinostroza, G.N., Ytrestøyl, T., Ruyter, B. and Bjerkeng, B. (2004) *Plasma appearance of unesterified astaxanthin geometrical E/Z and optical R/S isomers in men given single doses of a mixture of optical 3 and 3'R/S isomers of astaxanthin fatty acyl diesters* Comp. Biochem. Biophys. **Part C, 139**, 99-110
Osterlie, M., Bjerkeng, B. and Liaaen-Jensen, S. (2000) *Plasma appearance and distribution of astaxanthin E/Z and R/S isomers in plasma lipoproteins of men after single dose administration of astaxanthin* J. Nutr. Biochem., **11**, 482-490

Atherosclerosis risk

Bassendine, M., Nielsen, S. and Neely, D. (2016) *Hepatitis C virus (HCV) and atherosclerosis risk: a role for low-density immune complexes?* Atherosclerosis, **252**, e206

Bacteriochlorophylls, in

Dandler, J., Wilhelm, B. and Scheer, H. (2010) *Distribution of chlorophyll- and bacteriochlorophyll-derived photosensitizers in human blood plasma* Photochem. Photobiol., **86**, 182–193
Dandler, J., Wilhelm, B. and Scheer, H. (2010) *Photochemistry of bacteriochlorophylls in human blood plasma: Pigment stability and light-induced modifications of lipoproteins* Photochem. Photobiol., **86**, 331–341

CD36, binding to

Alkhatatbeh, M.J., Mhaidat, N.M., Enjeti, A.K., Lincz, L.F. and Thorne, R.F. (2011) *The putative diabetic plasma marker, soluble CD36, is non-cleaved, non-soluble and entirely associated with microparticles* J. Thromb. Haemost., **9**, 844–851

Diabetes Type 1

Ceriello, A., Kumar, S., Piconi, L., Esposito, K. and Giugliano, D. (2007) *Simultaneous control of hyperglycemia and oxidative sreess normalizes endothelial function in type 1 diabetes* Diabet. Care **30**, 649-654
Ceriello, A., Piconi, L., Esposito, K. and Giugliano, D. (2007) *Telmisartan shows an equivalent effect of vitamin C in further improving endothelial dysfunction after glycemia normalization in type 1 diabetes* Diabet. Care, **30**, 1694-1698

Ceriello, A., Esposito, K., Ihnat, M., Thorpe, J. and Giugliano, D. (2009) *Long-term glycemic control influences the long-lasting effect of hyperglycemia on endothelial function in type 1 diabetes* J. Clin. Endocrinol. Metab., **94**, 2751–2756

Diabetes Type 2

Anderson, R.A., Evans, M., Ellis, G. R., Graham, J., Morris, K., Jackson, S. K., Lewis, M. J., Rees, A. and Frenneaux, M. P. (2001) *The relationships between post-prandial lipaemia, endothelial function and oxidative stress in healthy individuals and patients with type 2 diabetes* Atherosclerosis, **154**, 475-483
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Dietary supplements (fish oil)

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Dietary supplements (garlic oil)

Dillon, S.A., Burmi, R.S., Lowe, G.M., Billington, D. and Rahman, K. (2002) *Antioxidant properties of aged garlic extract: an in vitro study incorporating human low density lipoprotein* Life Sci., **72**, 1538-1594
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Ghrelin

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Hedgehog proteins

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Hypercholesterolemia

Ruiu, G., Pinach, S., Veglia, F., Gambino, R., Marena, S., Uberti, B., Alemanno, N., Burt, D., Pagano, G., and Cassader, M. (2009) *Phytosterol-enriched yogurt increases LDL affinity and reduces CD36 expression in polygenic hypercholesterolemia* Lipids, **44**, 153–160

Hyperglycemia

Ceriello, A.C., Taboga, C., Tonutti, L., Quagliaro, L., Piconi, L., Bais, B., Da Ros, R. and Motz, E. (2002) *Evidence for an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial dysfunction and oxidative stress generation* Circulation, **106**, 1211-1218

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Hyperlipidemia

Hall, W.L., Jeanes, Y.M. and Lodge, J.K. (2005) *Hyperlipidemic subjects have reduced uptake of newly absorbed vitamin E into their plasma lipoproteins, erythrocytes, platelets, and lymphocytes, as studied by deuterium-labeled α-tocopherol biokinetics* J. Nutr., **135**, 58-63

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Isotachophoresis

Inano, K., Tezuka, S., Miida, T. and Okada, M. (2000) *Capillary isotachophoretic analysis of serum lipoprotein using a carrier ampholyte as spacer ion* Ann. Clin. Biochem., **37**, 708-716

LCAT deficiency

Yee, M.S., Pavitt, D.V., Richmond, W., Cook, H.T., McLean, A.G., Valabhji, J. and Elkeles, R.S. (2009) *Changes in lipoprotein profile and urinary albumin excretion in familial LCAT deficiency with lipid lowering therapy* Atherosclerosis **205**, 528–532

LDL binding to amyloid β

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LDL, lycopene levels

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LDL oxidation

Ganini, D. and Mason, R.P. (2014) *Absence of an effect of vitamin E on protein and lipid radical formation during lipoperoxidation of LDL by lipoxygenase* Free Radic. Biol. Med., **76**, 61–68

Helming, L., Winter, J. and Gordon, S. (2009) *The scavenger receptor CD36 plays a role in cytokine-induced macrophage fusion* J. Cell Sci., **122**, 453-459

Jantan, I. and Saputri, F.C. (2012) *Benzophenones and xanthones from Garcinia cantleyana var. cantleyana and their inhibitory activities on human low-density lipoprotein oxidation and platelet aggregation* Phytochemistry **80**, 58–63
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LDL, binding to proprotein convertase subtilisin/kexin type-9

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LDL, surface lipid modification

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Lipoprotein apheresis

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LOX-1 receptor

Vohra, R.S., Murphy, J.E., Walker, J.H., Homer-Vanniasinkam, S. and Ponnamabalam, S. (2007) *Functional refolding of a recombinant C-type lectin-like domaun containing intramolecular disulfide bonds* Protein Expr. Purif., **52**, 415-421

Lycopene, effects on LDL

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Metabolic syndrome

Mah, E., Sapper, T.N., Chitchumroonchokchai, C., Failla, M.L., Schill, K.E., Clinton, S.K., Bobe, G., Traber, M.G. and Bruno, R.S. (2015) *α-Tocopherol bioavailability is lower in adults with metabolic syndrome regardless of dairy fat co-ingestion: a randomized, double-blind, crossover trial* Am. J. Clin. Nutr., **102**, 1070–80

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Microparticles

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Mitotane binding

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Obestatin

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2b-5 Polychaete

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