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A rapid methodology for the isolation of intermediate-density lipoprotein: characterization of lipid composition and apoprotein content

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Abstract

Background: Intermediate-density lipoprotein (IDL) is a structurally related precursor of low-density lipoprotein (LDL). Although found in significantly lower levels, extensive evidence suggests that IDL shares LDL's capacity to promote atherosclerosis. To assist further investigation into IDL's composition and physiological relevance, we have established a rapid method to extract IDL from plasma.
Methods: IDL was isolated from plasma by sequential floatation ultracentrifugation in 3 h, a significantly shorter isolation time than previously published methods. Apoproteins (apo) B_{100}, CIII, and E, together with the level of albumin contamination, were quantified using single radial immunodiffusion. The lipid composition of IDL was measured using automated enzyme assays.
Results: The percent recovery of lipid from all lipoprotein fractions (VLDL+IDL+LDL+HDL) was 97.0±4.9% when compared to total plasma lipid. IDL had a reduced concentration of apo CIII, apo E, triglyceride, and free cholesterol, and had a higher concentration of apo B_{100}, cholesterol ester, and phospholipid when compared to VLDL. Pure IDL migrated in advance of LDL during agarose electrophoresis.
Conclusions: This rapid technique minimizes damage to the integrity of IDL and yields sufficient quantities to allow accurate assessment of composition and susceptibility to in vitro oxidation, and thus facilitates further investigation of IDL in the development of atherosclerosis.
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Keywords: Rapid ultracentrifugation; Lipid and apoprotein composition; Lipoproteins

Abbreviations: Apo, apoprotein; $CuCl_2$, copper II chloride; CVD, cardiovascular disease; IDL, intermediate-density lipoprotein; KBr, potassium bromide.

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1. Introduction

Ultracentrifugation is commonly used for the isolation of lipoproteins from plasma according to their hydrated density (*d*). Endogenous lipoproteins are classified into four main fractions: very-low-density lipoprotein VLDL (d<1.006 g/ml), intermediate-density lipoprotein (IDL) (*d*, 1.006→1.019 g/ml), low-density lipoprotein (LDL) (*d*, 1.019→1.063 g/ml), and high-density lipoprotein (HDL) (*d*, 1.063→1.21 g/ml). Exhaustive qualitative and quantitative research has been carried out on the most abundant lipoproteins (VLDL, LDL, and HDL), however, there have been relatively few publications on IDL. Investigations into IDL have been hindered, in part, due to its relatively low concentration in plasma and, to a greater extent, due to the lack of efficient methods for its isolation.

It is widely accepted that LDL has a positive association with the development of premature atherosclerosis, and although IDL is found in significantly lower levels than LDL, evidence suggests that IDL is also atherogenic. Epidemiological studies have identified elevated IDL as an independent risk factor for cardiovascular disease (CVD) [1–3]. This is supported by clinical studies, which have documented raised levels of IDL in individuals at greater risk of developing CVD, such as obese subjects and patients with type III hyperlipoproteinaemia [4,5], renal disease [6–9], or type 2 diabetes [10–12]. Indeed, it has been reported that changes to IDL levels are a better predictor of the severity and progression of atherosclerosis than changes to LDL or VLDL levels [13–15]. Evidence from the MARS study identified an independent association between the progression of coronary artery lesions and IDL, but found no link with total LDL [16]. It has been suggested that a proportion of the risk accredited to elevated LDL levels is, in fact, due to the inclusion of IDL in standard LDL measurements [2,14,17].

To further assist investigation into IDL composition and physiological relevance, we have established a rapid method to extract IDL from plasma. We describe a rapid, low-temperature sequential floatation method for the isolation of IDL; isolation was achieved in less than 3 h, a significantly shorter time than previously reported methods, the shortest of which can take in excess of 15 h. The centrifugation time was chosen to minimize ex vivo modifications of the lipoprotein particle, whilst the position of tube slicing for lipoprotein extraction ensured high yields. This rapid technique yielded pure IDL in sufficient quantities to facilitate full characterisation of the particle and also made possible studies to examine its oxidation potential and effect on cell function. We validated this methodology by comparing the lipid and apoprotein (apo) composition of IDL with previously documented results.

2. Materials and methods

2.1. Plasma separation

Fasting peripheral venous blood was obtained from healthy laboratory staff with no known history of hyperlipidaemia and collected into EDTA-coated tubes (EDTA 1.5 mg/ml); these participants shall be referred to as control subjects. Plasma was removed following centrifugation at 1100×*g* for 10 min at 4 °C within 30 min from venipuncture and stored in 2-ml aliquots at −70 °C. Lipoproteins were isolated by rapid ultracentrifugation from individual plasma samples and from a pooled plasma stock.

2.2. Preparation of density solutions

Solutions of the following density were prepared for overlaying during ultracentrifugation: 1.006, 1.019, and 1.063 g/ml. Normal saline (d=1.006 g/ml; 0.196m, 11.42 g NaCl+1 l H_2O) was adjusted to desired density by the addition of calculated amounts of potassium bromide (KBr).

The formula for adjusting solutions to the required density is as follows:

$$\text{Weight of KBr salt} = \frac{V(d_2 - d_1)}{1 - vd_2}$$

where V is the volume of solution, d_1 is the starting density, d_2 is the desired density, and v is (0.2697) the partial specific volume of the solution with d=1.006 g/ml at 5 °C [18]. The density of each solution was verified by measurement on a Digital Densitometer (DMA 35; Paar Scientific) and solutions were spiked to contain 0.1% EDTA to minimize oxidation of lipoprotein particles.

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