

Validation of a Method to Prepare Artificial Chylomicron Remnant-like Particles

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Chylomicron remnants are atherogenic particles that can have access to the endothelial space, where they can initiate the atherogenic process. Isolation of these particles from serum for experimental assays presents several technical difficulties, including contamination of other lipoproteins and ethical issues. For that reason, the creation of artificial chylomicron remnant-like particles (CRLP) has resulted a useful tool in studies of postprandial metabolism. However, the methodology for CRLP preparation has not been adequately validated, due to the lack of appropriate techniques. The present work was aimed to test the reproducibility of the methodology for CRLP formation.

CRLP were prepared by sonication of a mixture of trilinolein, cholesteryl oleate, cholesterol and phospholipids classes in 0.9% NaCl in Tris buffer (20 mM, pH 7.4). Lipid fractions with a Svedberg flotation rate $Sf < 20$, $20 < Sf < 60$, $60 < Sf < 400$ and $Sf > 400$ were collected by density gradient ultracentrifugation. Lipids were extracted using chloroform:methanol (2:1), and the lipid classes content determined by HPLC coupled to a light-scattering detector (ELSD). The particle size of the $60 < Sf < 400$ and $Sf > 400$ fractions was measured by means of Dynamic Light Scattering using a Zetasizer Nano ZS (Malvern Instruments Ltd.) instrument. For validation purposes, both intra- and inter-assay analyses were performed.

The highest lipid content was found in the $60 < Sf < 400$ fraction collected. The HPLC-ELSD analysis of CRLP revealed that the lipid composition of the $60 < Sf < 400$ fraction was the closest to the starting mix. The highest triglyceride concentration and the lowest phospholipid concentration were found in the $Sf > 400$ fraction. Conversely, the lowest triglyceride concentration and the highest phospholipid concentration were found in the $Sf < 20$ fraction. The mean size of the $60 < Sf < 400$ fraction was 195.1 nm while that of the $Sf > 400$ was 347.8 nm. The area under the curve for the particle size distribution of the $60 < Sf < 400$ fraction was lower than that of the $Sf > 400$ fraction, showing a more homogeneous range of particle size.

In conclusion, the $60 < Sf < 400$ fraction showed a close resemblance to what it was expected to obtain when preparing CRLP in terms of lipid composition and particle size. Therefore, we consider that this fraction can be used as an artificial model of chylomicron remnants for metabolic studies *in vitro*.