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Commonly used method for chylomicron isolation catches less than 0.1% of chylomicrons from whole plasma

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Abstract

Health effects of orally ingested bioactive compounds can only occur if bioactive molecules are absorbed and transported to target tissues. Paradoxically, many foods are rich in micronutrients but intestinal absorption is often limited. Immediately after absorption, lipid soluble nutrients are packaged into chylomicrons (CM), therefore quantification of the micronutrient content in CM has been used as tool to evaluate bioavailability[1]. For bioavailability studies the isolated CM fraction must not contain liver derived lipoproteins (VLDL, LDL) since only intestine derived CM carry recently absorbed lipids and lipophilic micronutrients. As isolated CM can be contaminated with liver derived lipoproteins, this study evaluated the purity of collected CM and VLDL fractions. Each CM contains one apolipoprotein B-48 (apoB-48) and each VLDL contains one apolipoprotein B-100 (apoB-100) on its surface[2] therefore purity of CM and VLDL fractions was evaluated via the presence/absence of apoB-100 and apoB-48.

CM and VLDL fractions were isolated as previously described[1,3]from whole plasma collected at 0, 2, 4 or 6 h after participants consumed a lipid (15.4g) and carotenoid (36mg) rich smoothie (480mL). Plasma density was adjusted to 1.1g/mL with KBr (0.14g/mL), placed in ultracentrifugation tubes (38 mL, thickwall) and overlaid with 3 solutions containing NaCl, KBr and Na-EDTA with densities of 1.020, 1.065 and 1.006g/mL.CM fraction was collected after ultracentrifugation at 25,000 rpm for 34 min in a Beckman Coulter Optima XE-90 with SW32Ti swinging bucket rotor. VLDL fraction was collected after additional 102 min at 25,000 rpm.

ELISA analysis revealed that apoB-48 and apoB-100 were present in both CM and VLDL fractions. Less than 0.1% of plasma apoB-48 (< 98pmol/L) was present in CM or VLDL fraction. Less than 0.3% (< 2,425pmol/L) and 4% (< 54,124pmol/L) of plasma apoB-100 was present in CM and VLDL fractions, respectively. Low recoveries of apoB-48 and apoB-100 in CM and VLDL fraction suggests that ultracentrifugation neither concentrated nor isolated CM or VLDL. While ourapoB-48 and apoB-100 results agree with previously reported values in plasma, CM and VLDL[3,4], no previous study evaluated apoB-48 and apoB-100 recovery from plasma.

Conflict of Interest

There is no conflict of interest

References

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