Proceedings of the Nutrition Society (2010), **69 (OCE1)**, E140 doi:10.1017/S0029665109993284

Over- and undernutrition: challenges and approaches. 29 June–2 July 2009

Short-term storage of plasma at 4°C, or at −80°C, has little impact on TAG-rich lipoprotein composition

V. Calabuig-Navarro[1], C. M. Walden[2], K. G. Jackson[1] and A. M. Minihane[1]
[1]*Department of Food Biosciences, University of Reading, Reading RG6 6AP, UK and* [2]*Unilever Discover, Colworth Science Park, Sharnbrook MK44 1LQ, UK*

The concentration of fasting and postprandial TAG levels and TAG-rich lipoprotein (TRL) composition are increasingly recognised as being important determinants of atherogenesis and CHD risk[(1)]. TRL include chylomicrons (CM), VLDL1 and VLDL2, which are typically separated by ultracentrifugation techniques. Although TRL are often separated from plasma samples that have been stored for considerable periods of time at 4°C, there is currently a distinct lack of information on the impact of storage on TRL composition or cellular uptake. The current pilot study was conducted to address this knowledge deficit.

Nine healthy men (mean age 40 (SD 25) years and BMI 25 (SD 3) kg/m^2) gave a fasting blood sample before consuming a test meal containing 50 g fat. After 5 h a further blood sample was taken. Once the plasma was collected the TRL (Svedberg flotation rate (S_f) >400, CM-rich fraction; S_f 60–400, VLDL1-rich fraction; S_f 20–60, VLDL2-rich fraction) were isolated using density-gradient ultracentrifugation either immediately (day 0) or the plasma was stored for 3 or 7 d at 4°C or 30 d at −80°C. Lipids (cholesterol and TAG) within each of the TRL fractions were measured using automated colorimetric assays (Instrumentation Laboratories, Warrington, UK) and apoB, apoC-III and apoE using immunoturbidimetric assays (Alpha Laboratories, Eastleigh, Hants., UK). The particle size of the TRL fractions were analysed using a N4 Plus Sub Micron Particle Sizer (Beckman Coulter, High Wycombe, Bucks., UK).

Compared with the fresh sample (day 0), there were no differences in the lipid or apo composition of TRL stored at 4°C for 3 d or at −80°C for 30 d. Differences were observed in the composition of the TRL stored >3 d at 4°C, with 504% ($P<0.001$) and 301% ($P<0.001$) higher cholesterol and apoB levels in the fasting VLDL1-rich fraction stored at 7 d relative to 0 d. Comparable 211% ($P<0.001$) and 84% ($P=0.001$) higher cholesterol and apoB were observed for the VLDL2-rich fractions. A large impact of storage at 4°C for 7 d on TRL composition was also evident for the postprandial (5 h) samples. Studies examining the impact of plasma storage on receptor-mediated uptake of TRL by hepatocytes (HepG2) are currently ongoing.

The results of the present pilot study indicate that TRL derived from plasma stored at −80°C and 4°C for ≤3 d had a comparable composition to freshly-isolated TRL. However, 7 d of storage at 4°C significantly impacts on the lipid and apo composition of the TRL particles within the VLDL1 and VLDL2 fractions.

Storage at −80°C has been chosen for the main trial, which is designed to examine the combined impact of apoE genotype and dietary fat composition on fasting and non-fasting plasma lipid, apo, TRL composition and binding and uptake of TRL and LDL by hepatocytes in culture.

The PhD programme is jointly sponsored by The University of Reading, Research Endowment Trust Fund and Unilever Discover.

1. Nordestgaard BG, Benn M, Schnohr P *et al*. (2007) *JAMA* **298**, 299–308.