Analytical approach for the measurement of oxidized lipids and medium-chain fatty acids: Emerging lipids in human health and nutrition

Lipids and lipoproteins play a key role in the pathogenesis of coronary heart disease (CHD). Despite the strong association of oxidized lipoprotein with CHD, there is a paucity of evidence that details the chemistry and existence of molecular species of oxidized lipids in human plasma and native lipoproteins. Identification and quantification of oxidized lipids may provide valuable information to understand the process of atherogenesis and aid in CHD risk stratification. Therefore, this study aimed to scrutinize cholesteryl ester hydroperoxides (CEOOH) that can exist in plasma and unmodified lipoproteins. Furthermore, the role of triglyceride, which is predominantly transported by the triglyceride-rich lipoproteins (TRL), in the progression of atherosclerosis is uncertain. However, identification of oxidized triglycerides and their possible association with atherosclerosis may provide new insight to understand the pathogenicity of TRL. Therefore, this study is also focused on the qualitative and quantitative analysis of triglyceride hydroperoxides (TGOOH). Moreover, a condition known as triglyceride deposit cardiomyovascularopathy (TGCV), characterized by massive accumulation of triglycerides in the coronary atherosclerotic lesions, has improved clinical outcomes after dietary therapy of medium-chain triglycerides (MCT); therefore, this study also aimed to develop a quantitative assay for the measurement of capric acid (FA10:0), a medium-chain fatty acid, which may be useful in the therapeutic monitoring of such therapy.

For the analysis of CEOOH, fasting plasma was collected from six healthy volunteers. Very-low density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL) were isolated from the plasma by sequential ultracentrifugation. The lipid extract of the plasma and lipoproteins were subjected for the determination of CEOOH using liquid chromatography/LTQ ion trap mass spectrometry (LC/LTQ Orbitrap). A total of six molecular species of CEOOH, namely Ch18:1-OOH, Ch18:2-OOH, Ch18:3-OOH, Ch20:4-OOH, Ch20:5-OOH, and Ch22:6-OOH, were identified on the basis of their mass spectra and retention time on the LC. Of them, Ch18:2-OOH, Ch20:4-OOH, Ch20:5-OOH, and Ch22:6-
OOH were detected in all IDL samples, while only Ch22:6-OOH was detected in all VLDL samples. Except for Ch18:3-OOH, all CEOOH species were also detected in the plasma, with constant detection of Ch20:5-OOH, and Ch22:6-OOH in all plasma samples.

Similarly, the analysis of TGOOH was performed in the fasting plasma of nine human volunteers. VLDL and IDL were separated from the plasma (n=6) by sequential ultracentrifugation, followed by the isolation of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) using size-exclusion high-performance liquid chromatography (HPLC). LC/LTQ Orbitrap analysis of the lipid extract revealed 11 molecular species of TGOOH in either plasma or VLDL and IDL, of which TGOOH-18:1/18:2/16:0, TGOOH-18:1/18:1/16:0, TGOOH-16:0/18:2/16:0, TGOOH-18:1/18:1/18:1, and TGOOH-16:0/20:4/16:0 were most dominant. TGOOH-18:1/18:1/16:0 and TGOOH-16:0/18:2/16:0 were present in all plasma. Mean concentration of plasma TGOOH was 56.1±25.6 µmol per mol of triglycerides. These TGOOH molecules were carried by VLDL and IDL but not by LDL and HDL. Although IDL is relatively poor in triglycerides, the mean concentration of TGOOH in it was higher than in VLDL (512.5±173.2 vs 349.8±253.6 µmol/mol triglycerides).

Lastly, the quantitative analysis of FA10:0 was performed in blood samples taken from healthy Japanese volunteers who were in fasting (n=5, male/female=3/2, age 31±9.3 years old) and non-fasting (n=106, male/female=44/62, age 21.9±3.2 years old) states, using HPLC after derivatization with 2-nitrophenylhydrazine. This assay is analytically simple, rapid, and sensitive that specifically measures FA10:0. The inter- and intra-assay coefficient of variation of FA10:0 assay ranged from 1.7–3.9% and 1.3–5.4%, respectively, with an analytical recovery of 95.2–104.0%. FA10:0 was not detected in the fasting plasma samples. Of the plasma collected during the non-fasting state, 50 samples (47%) lacked detectable amounts of FA10:0, while the remaining samples had negligible amounts of FA10:0, with the mean of 0.3 µmol/L (SD–0.4, Max–1.6).

In conclusion, this study revealed that human plasma contains several molecular species of CEOOH and TGOOH. VLDL and IDL carry these oxidized lipids in the plasma. The existence of CEOOH and TGOOH in the TRL is possibly associated with its atherogenicity. The clinical utility of measuring CEOOH and TGOOH in these lipoproteins needs to be investigated for risk assessment of cardiovascular disease. Additionally, the human plasma contains trace amounts of FA10:0 during a non-fasting state, which may reflect dietary MCFA that have escaped the hepatic utilization. This assay can be used for monitoring the concentration of FA10:0 during dietary therapy with MCT.